Macroecology of

New Zealand Ephemeroptera

A thesis submitted in partial fulfilment of the requirements for

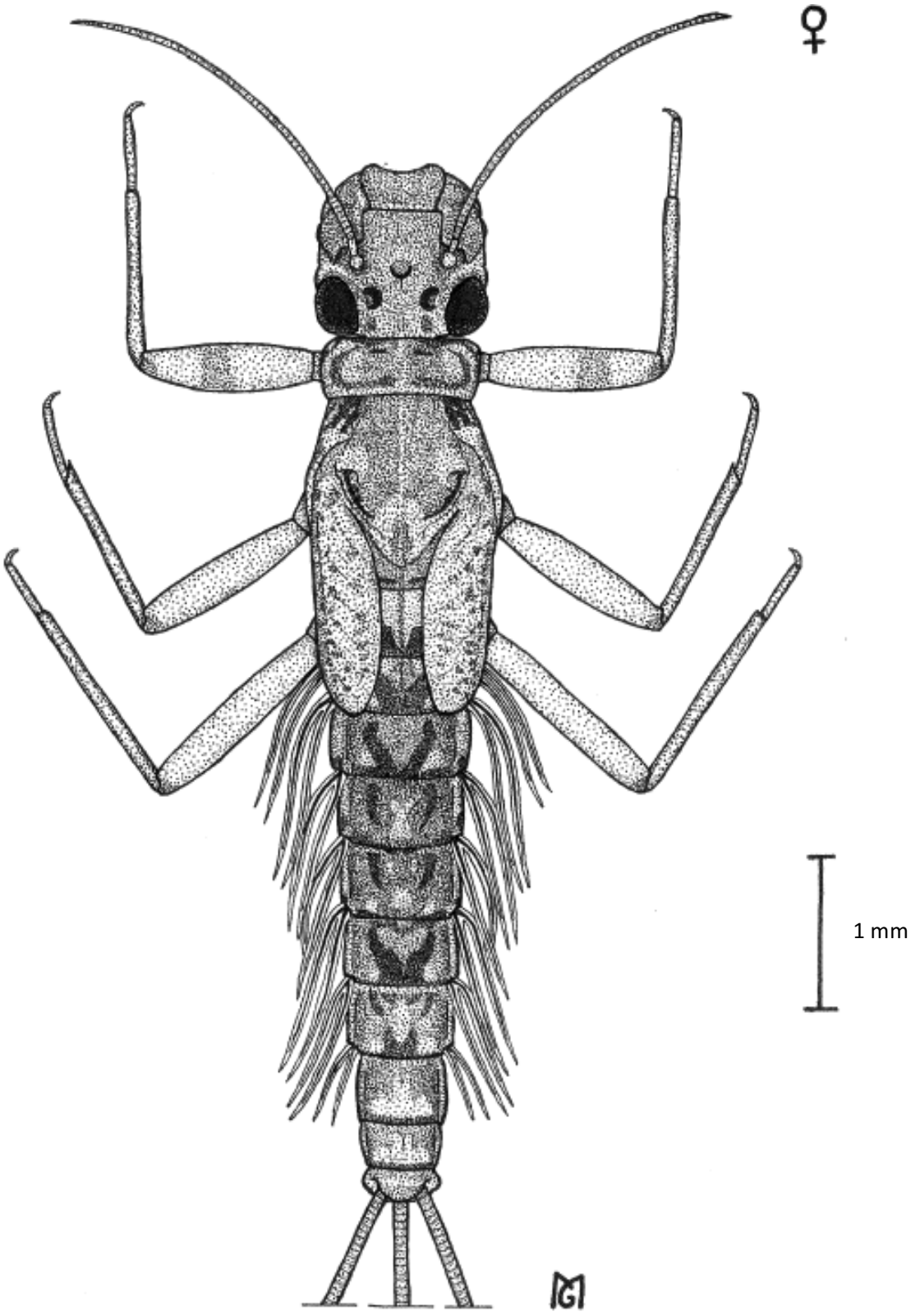
the Degree of Doctor of Philosophy in Ecology

by

Stephen Robert Pohe

University of Canterbury

April 2019



Late instar nymph of *Aupouriella pohei* Winterbourn, 2009 – New Zealand's rarest mayfly.

Thesis abstract

The aim of this thesis is to extend our knowledge of the New Zealand Ephemeroptera (mayflies) with particular reference to the latitudinal and altitudinal distribution of species and community assemblages. The study was centred on a nationwide survey of 81 streams located in predominantly unmodified landscapes, many of which were native forest. Three sites on each stream were sampled by light trapping of imagos (adults) and subimagos as well as supplementary collecting of nymphal stages. An initial study of four types of fluorescent lights showed that although mayflies were attracted to all four types, blacklight and blacklight-blue light were most effective. Because, blacklight-blue light was also less effective at attracting non-target terrestrial species, it was used in the nationwide survey. A total of 47 described mayfly species were obtained in the survey, 85% of the fauna known to occur in the country. Five probable undescribed species were also collected, and several previously described species were recognised as possible 'morphospecies' complexes, that may contain cryptic species. Of the almost 14,000 individuals taken in light traps, 39% were imagos and 61% subimagos; 63% of imagos were female, whereas the sex ratio of subimagos was approximately even (54% male, 46% female). Three gynandromorph adults were also collected. Alpha diversity throughout the country ranged from 3–24 species per stream. Species richness declined from north to south, and with increasing altitude, and the similarity of mayfly assemblages to each other declined with distance. Thirty-eight species are recognised from the North Island (15 North Island endemics), 41 from the South Island (16 South Island endemics) and 23 shared by both islands. Species richness on Stewart Island was 14 (1 possible endemic) but further study of the island's fauna is required. Effects of latitude and altitude were revealed in the widespread and common species *Coloburiscus humeralis*, which increased in size (forewing length) from north to south, and with altitude. Investigations focusing on two poorly known species, *Siphlaenigma janae* and *Isothraulus abditus*, showed they had restricted and fragmented distributions, and were in need of habitat protection to reduce the likelihood of further decline. Genetic structure of *S. janae*, assessed using the barcoding fragment of the mitochondrial COI gene, indicated the presence of geographically distinct haplotype networks in the northern and central North Island, and the northwest South Island. *Isothraulus abditus*, which was found only in the North Island from Northland to Whanganui, exhibited less genetic variability (COI gene) than *S. janae*, but also had geographically isolated haplotypes, suggesting limited dispersal. Both species had their greatest genetic diversity in Northland, which other workers have suggested was a likely refuge from Pleistocene glaciations and subsequently a source of recolonisation further south. Overall the present study has expanded knowledge of the New Zealand Ephemeroptera by documenting patterns of species richness and community similarity in relation to latitude and altitude within the country, and provided new information and a better understanding of the distribution, ecology and conservation needs of poorly known species.

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Chapter 2 - Summarising the New Zealand mayfly fauna: an annotated checklist.

Publication: Pohe, SR. 2018. An annotated checklist of New Zealand mayflies (Ephemeroptera), 2018. New Zealand Natural Sciences 43: 1–20.

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Contribution overview: Pohe (95%), Winterbourn (5%).

This was a sole-author paper by the Candidate but Michael Winterbourn was offered co-authorship for his contributions (but requested acknowledgment instead of co-authorship).

Stephen Pohe (Candidate): Lead all aspects of the study; designed the study in consultation with Michael Winterbourn; carried out all fieldwork and identified all specimens; obtained, generated and analysed all the data; wrote the manuscript with edits by Michael Winterbourn; constructed all figures; dealt with manuscript submission, revision and proofs.

Certification by Co-authors:

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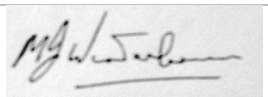
Name: ***Michael Winterbourn*** Signature:  Date: *01.02.2019*

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Chapter 3 - What fluorescent lights are best to attract mayflies to light traps for biodiversity surveys?

Publication: Pohe, SR, Winterbourn, MJ, Harding, JS. 2018. Comparison of fluorescent lights with differing spectral properties on catches of adult aquatic and terrestrial insects. New Zealand Entomologist 41: 1–11.

Please detail the nature and extent (%) of contribution by the candidate:

Contribution overview: Pohe (80%), Winterbourn (15%), Harding (5%).

Stephen Pohe (Candidate): Carried out all aspects of the study (overseen by Jon Harding); designed the study in consultation with supervision from Michael Winterbourn and Jon Harding; carried out all fieldwork; identified all specimens; analysed the data with supervision of statistical consultants Daniel Gerhard and Elena Moltchanova and Lecturer Helen Warburton; wrote the manuscript; constructed all tables and figures; dealt with publication submission and manuscript revision with all authors editing the draft manuscript and checking the journal proof.

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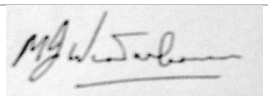
Name: ***Michael Winterbourn*** Signature:  Date: *01.02.2019*

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Chapter 5 - Ecology and conservation of Siphlaenigma janae.

Publication: Pohe, SR, Winterbourn, MJ, Goldstien, SJ, Harding, JS. 2018. Distribution, body size, genetic structure and conservation of *Siphlaenigma janae* (Insecta: Ephemeroptera). New Zealand Journal of Zoology 45: 154–170.

Please detail the nature and extent (%) of contribution by the candidate:

Contribution overview: Pohe (60%), Winterbourn (20%), Goldstien (15%), Harding (5%).

Stephen Pohe (Candidate): Lead all aspects of the study (overseen by Jon Harding); designed the study with supervision from Michael Winterbourn; carried out all fieldwork with contributions by Michael Winterbourn and Jon Harding; identified all specimens and compiled all distribution data; carried out all molecular lab-work and statistical analyses under direct supervision of Sharyn Goldstien; wrote the manuscript with contributions from Michael Winterbourn and Sharyn Goldstien; constructed all tables and figures; dealt with publication submission and manuscript revision with all authors editing the draft manuscript and checking the journal proof.

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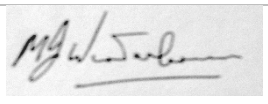
Name: ***Michael Winterbourn***	Signature:		Date: *01.02.2019*
Name: ***Sharyn Goldstien***	Signature:		Date: *10.04.2019*
Name: ***Jon Harding***	Signature:		Date: *13 Feb 2019*

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Please indicate the chapter/section/pages of this thesis that are extracted from co-authored work and provide details of the publication or submission from the extract comes:

Chapter 6 - Ecology and conservation of Isothraulus abditus.

Publication: Pohe, SR, Winterbourn, MJ, Goldstien, SJ, Ball, OJ-P, Harding, JS. 2019. Distribution, nymphal habitat, genetic structure and conservation of the New Zealand mayfly *Isothraulus abditus* (Insecta: Ephemeroptera) and a description of its subimago. New Zealand Journal of Zoology 46: 13–30.

Please detail the nature and extent (%) of contribution by the candidate:

Contribution overview: Pohe (60%), Winterbourn (20%), Goldstein (10%), Ball (9%), Harding (1%).

Stephen Pohe (Candidate): Lead all aspects of the study (overseen by Jon Harding); designed the study in consultation with supervision from Michael Winterbourn and Olly Ball; carried out all fieldwork with contributions by Olly Ball; identified all specimens; carried out all molecular lab-work and statistical analyses with supervision of Sharyn Goldstein; wrote the manuscript with contributions from Michael Winterbourn and Sharyn Goldstein; constructed all tables and figures; dealt with publication submission and manuscript revision with all authors editing the draft manuscript and checking the journal proof.

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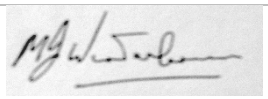
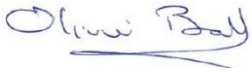
Name: ***Michael Winterbourn***	Signature:		Date: *01.02.2019*
Name: ***Sharyn Goldstien***	Signature:		Date: *10.04.2019*
Name: ***Olly Ball***	Signature:		Date: *28.01.2019*
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Please indicate the chapter/section/pages of this thesis that are extracted from co-authored work and provide details of the publication or submission from the extract comes:

Appendix 3 - Aquatic invertebrate communities of Little Barrier Island Nature Reserve.

Publication: Pohe, SR, Wade, L, Winterbourn, MJ, Ball, OJ-P. 2019. Invertebrate fauna of ephemeral streams on Hauturu-o-Toi/Little Barrier Island in northern New Zealand. New Zealand Journal of Zoology. https://doi.org/10.1080/03014223.2019.1576214

Please detail the nature and extent (%) of contribution by the candidate:

Contribution overview: Pohe (50%), Wade (10%), Winterbourn (30%), Ball (10%).

Stephen Pohe (Candidate): Lead all aspects of the study; designed the study in consultation with Lyn Wade and with supervision from Michael Winterbourn and Olly Ball; carried out all fieldwork equally (as a team) with Lyn Wade and Olly Ball; identified all specimens with contributions from Lyn Wade and Michael Winterbourn; analysed the data with supervision of Michael Winterbourn and Olly Ball; wrote the manuscript in conjunction with Michael Winterbourn (50/50); constructed tables and figures with contributions from Michael Winterbourn and Olly Ball; dealt with publication submission and manuscript revision; all authors edited the draft manuscript and checked the journal proof.

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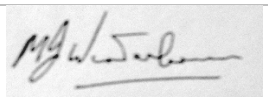
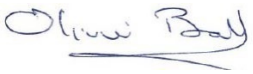
Name: ***Michael Winterbourn***	Signature:		Date: *28.01.2019*
Name: ***Olivier Ball***	Signature:		Date: *28.01.2019*
Name: ***Lyn Wade***	Signature:		Date: *31.01.2019*

General acknowledgements

I first and foremost acknowledge my university supervisors. To Professor Jon Harding, for his guidance and contributions to the thesis, for dealing with behind-the-scenes paperwork that is a necessary evil in academia, and particularly for encouraging me to include a 'wildcard' component in the thesis — an element that was well outside of my comfort zone and skill-set. For this we chose to include a molecular element, using DNA analyses to confirm species designations, and population genetics to assess genetic variability of select species. I am very much a field ecologist and prior to this study, I could barely spell DNA, so while I concede I will never be a molecular ecologist, I acknowledge I have discovered a whole new world to biology, learnt a range of new skills, and an appreciation for the lab-based sciences. To Dr Sharyn Goldstien, who took me on despite knowing molecular ecology was new to me, and has guided me through the minefield of chemicals, concentrations, extractions and sequencing. I am someone who likes to know 'why' but have learnt that in genetics, sometimes "it just does". I thank Sharyn most of all for her patience, for her willingness to help well after most academics had gone home, and also for her genuine concern for providing the best outcomes possible, for all of her students. Finally, to Emeritus Professor Michael Winterbourn whose calm and collective manner, eagerness to discuss ideas, phenomenal editing and feedback abilities, and collegial friendship, have smoothed the way on what could have been a rocky journey. When I wasn't out in the field "chasing bugs" it would be unusual not to find us in the lab peering down microscopes and arguing over identifications, although coffee breaks did slow productivity on occasion. For the time Mike as given to help develop my scientific skills and critical thinking, and for helping me to see the forest through the trees, I owe a huge acknowledgement, one that can't be quantified or put into words. And also an apology, for tasks that took me ten times longer than they should have; I will always be a details man, but my efficiency has improved… somewhat!

I also thank my long-time mentor, colleague and friend, Dr Olivier Ball, for all his contributions and support throughout my BSc, MSc and PhD. Now the monkey's off my back we can go sailing and fishing again! To Ray Tana, for his continued support, discussions about statistics and good feeds of paua, mussels and steak... and scallop season is not too far off now bro – time to visit the Kaipara. And to Lyn Wade, a big thank you for facilitating the greatest field-trip ever to Little Barrier Island. I also acknowledge Emeritus Professor Douglas Craig and Dr Trevor Crosby for providing Figure 4.5, Professor Ian Hogg for facilitating access to the Barcode of Life Data System, and Dr Shelley Ball for providing a genetic sequence of *Coloburiscus humeralis*. My thanks and appreciation also go to all the collections staff at Otago, Canterbury and Te Papa (New Zealand) Museums for their assistance during collection visits, and a special acknowledgment to Matthew Shaw (Canterbury Museum) for all his efforts.

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I express my appreciation to all the Biological Sciences staff, and in particular Linda Morris, Hayley Devlin, Kim Roberts, Jan McKenzie, Alan Woods, Nick Etheridge, Matthew Walters, Nicole Lauren-Manuera and Nicki Judson, for their technical support, but also for their encouraging words during trying times. A big thank you also to ConSERT Associate Professor's Tammy Steeves and Pieter Pelser for welcoming me into their lab-group and providing a supportive learning environment. Thank you also to my UoC peers, Channell Thoms, Elizabeth Graham, Frank Burdon, Helen Warburton, Justin Pomeranz and Mark L. Galatowitsch, for discussions about the answers to the ultimate PhD questions of life, the universe, and everything (namely ecology, stats, and funding). Also, to Helen Warburton, Raymond Tana and Daniel Gerhard for providing valuable tutoring and advice on statistical design and analyses, to Daisy Stainton, Rachel van Heugten and members of ConSERT for advice on technical molecular issues, and to Mark L. Galatowitsch who was employed to assist with photography for body size measurements in Chapter 4, and also commissioned to produce mayfly illustrations for a future publication. An example of Mark's talent is presented at the beginning of this thesis (Page 2).

The Department of Conservation, Auckland Regional Council, Gisborne District Council and Queen Elizabeth II National Trust provided collecting permits or written approvals to undertake research on their respective administered lands. The following kaitiaki also granted approval for site access and specimen collection: Te Rūnanga o Whaingaroa, Te Rūnanga o Te Rarawa, Puketi Forest Trust, Te iwi o Ngati Wai, Te iwi o Te Roroa, Pukenui Western Hills Forest Trust, Nga Ahi Kaa, Te Uri o Hau, Little Barrier Island Supporters Trust, Ngati Manuhiri, Tūhoe Te Mana Motuhake mai Waikaremōana Tribal, Ngāi Tamanuhiri Whanui, Eastern Taranaki Environmental Trust, Rangitaane o Manawatu, Te Rūnanga o Ngai Tahu, Maurice White Forest Trust, and five private landowners. The nationwide survey was successful with the support of these groups, who also offered valuable local knowledge, accommodation and assistance in the field, all of for which I am immensely grateful.

Table of contents

Thesis preface

I have been fascinated in the mayfly fauna since my first encounters as an undergraduate student. It was initially a love–hate affair as, although these unique insects intrigued me, trying to identify them seemed impossible. The relationship continued after I graduated and set up a business in the north of New Zealand monitoring river health for regional councils and private companies, using aquatic invertebrates as biological indicators. My interest in the Ephemeroptera was amplified further when I discovered a new genus of mayfly in the far north of New Zealand, which was formally described bearing my family name (*Aupouriella pohei*, figured on Page 2). But as this research will show, my early frustrations were justified as northern mayfly communities have the greatest diversity in the country. In addition, body size, and a number of other morphological characters important in mayfly identification, can be variable in populations across the country. Further, it appears there are quite a number of cryptic, undescribed species. Describing them may be the next step…

Stephen R. Pohe, April 2019.

Chapter 1 – *General introduction*

"An understanding of the natural world, and what's in it,

is a source of not only a great curiosity, but great fulfilment".

Sir David Attenborough, 2004.

A high-elevation stream site in the Southern Alps; a location where several uncommon mayfly species were found.

New Zealand Ephemeroptera

Mayflies are insects of the order Ephemeroptera, a name derived from the Greek words *ephemeros* meaning short-lived and *pteron* meaning wing. Short-lived refers specifically to the winged adult stage that lives only a few days. There are approximately 3,600 species described globally (Pohe 2018) and they constitute a well-supported monophyletic insect group (Ogden & Whiting 2003, 2005). Mayflies are considered to be the most primitive of the extant winged insects, with a lineage dating back over 300 million years (Grimaldi & Engel 2005; Bauernfeind & Soldán 2012). Ephemeroptera are present on all continents except Antarctica, but many families and genera have strong distributional affinities to particular biogeographical regions. Only three families have global distributions; the remaining 39 are fairly evenly distributed between the northern and southern hemispheres (Barber-James et al. 2008). The New Zealand fauna consists of 57[†] described species that belong to 20 genera, in eight families (Pohe 2018). All species and genera are endemic to the New Zealand Region, as are three families (Ichthybotidae, Rallidentidae, Siphlaenigmatidae). Four other families with New Zealand representatives, Ameletopsidae, Coloburiscidae, Nesameletidae and Oniscigastridae, have amphinotic distributions (Gondwanan origins shared with the austral continents of South America and Australia) and one, Leptophlebiidae, has a global distribution (Table 1.1). A detailed account of the taxonomic history of New Zealand's mayfly fauna, and a formal checklist, are presented as Chapter 2.

The nymphal (juvenile) stages of mayflies are aquatic and typically restricted to unpolluted freshwaters. They generally take one year to develop (see Scarsbrook 2000, and references within), but in some cases as many as three years (Domínguez et al. 2006; Macfarlane et al. 2010). As they grow they moult an indeterminate number of times (most in the range 12–45) (Gullan & Cranston 2014). Globally, nymphs of many mayfly species occupy flowing streams and rivers, although some also occur in standing waters (lakes and ponds). In New Zealand, all species are primarily confined to streams and rivers, although they have occasionally been recorded in stony littoral zones of alpine lakes margins where there is regular wave action (Winterbourn & Lewis 1975; Greig 1976). The mayfly imago (adult) is a terrestrial insect characterised by small hindwings and larger triangular-shaped forewings that are held vertically over the body when at rest (Berner & Pescador 1988). Imagos typically live only a few days, although some live for only a few hours and others up to 14 days (Brittain 1982). Their main functions are dispersal and reproduction. Mayflies also have a unique intermediate life stage, the subimago, which is found between the nymphal and imaginal stages. It generally lasts a day and is distinguished by its dull appearance and hair-covered wings, which assist its emergence from the water (Edmunds & McCafferty 1988).

[†] Hitchings and Hitchings (2018) recently described two new species of New Zealand Ephemeroptera, however their very recent description has meant they are not included in analyses made in this thesis.

Table 1.1 The number of described mayfly species per family in the New Zealand, Australian, and South American (Gondwanan) regions, contrasted with the rest of the world. "Other regions" comprise all the biogeographical realms of Barber-James et al. (2008), other than New Zealand, Australia and South America.

	Family	New Zealand region[1]	Australian region[2]	South America[3]	All other regions[4]	Total
New Zealand fauna	Ameletopsidae	1	3	2	0	6
	Coloburiscidae	2	3	1	0	6
	Ichthybotidae	2	0	0	0	2
	Leptophlebiidae	39	80	214	384	717
	Nesameletidae	5	1	1	0	7
	Oniscigastridae	3	4	3	0	10
	Rallidentidae	2	0	0	0	2
	Siphlaenigmatidae	1	0	0	0	1
Non-New Zealand fauna	Acanthametropodidae	0	0	0	3	3
	Ameletidae	0	0	0	60	60
	Ametropodidae	0	0	0	2	2
	Austremerellidae	0	1	0	0	1
	Baetidae	0	19	171	794	984
	Baetiscidae	0	0	0	12	12
	Behningiidae	0	0	0	6	6
	Caenidae	0	12	30	211	253
	Coryphoridae	0	0	1	0	1
	Dicercomyzidae	0	0	0	4	4
	Dipteromimidae	0	0	0	2	2
	Ephemerellidae	0	0	0	150	150
	Ephemeridae	0	0	3	75	78
	Ephemerythidae	0	0	0	4	4
	Euthyplociidae	0	0	8	16	24
	Heptageniidae	0	0	0	605	605
	Isonychiidae	0	0	0	34	34
	Leptohyphidae	0	0	101	60	161
	Machadorythidae	0	0	0	1	1
	Melanemerellidae	0	0	1	0	1
	Metretopodidae	0	0	0	11	11
	Neoephemeridae	0	0	0	15	15
	Oligoneuriidae	0	0	25	39	64
	Palingeniidae	0	0	0	33	33
	Polymitarcyidae	0	0	73	29	102
	Potamanthidae	0	0	0	24	24
	Prosopistomatidae	0	1	0	26	27
	Pseudironidae	0	0	0	1	1
	Siphlonuridae	0	0	0	48	48
	Siphluriscidae	0	0	0	1	1

Family	New Zealand region[1]	Australian region[2]	South America[3]	All other regions[4]	Total
Teloganellidae	0	0	0	2	2
Teloganodidae	0	0	0	25	25
Tricorythidae	0	0	0	39	39
Vietnamellidae	0	0	0	3	3
Total	**55**	**124**	**634**	**2719**	**3532**

[1] Source: An annotated checklist of New Zealand mayflies, 2018 (Pohe 2018, see Chapter 2).
[2] Sources: Catalog of the Australian mayflies (Hubbard et al. 2006); Suter unpublished data, May 2018.
[3] Sources: Checklist of South American species of Ephemeroptera (Domínguez et al. 2012); Sartori & Barber-James unpublished data, May 2018.
[4] Sources: World checklist of freshwater Ephemeroptera species (Barber-James et al. 2013); Sartori & Barber-James unpublished data, May 2018.

The guts of mayfly subimagos and imagos are non-functional and the mouthparts are vestigial so they cannot feed. In contrast, the nymph is the feeding and growth stage of all species. The feeding ecology of New Zealand mayflies has been summarised by Winterbourn (2000). Many mayfly nymphs are grazers or collector-grazers that ingest algae, fine detrital particles and associated micro-organisms such as fungi and bacteria from the surfaces of hard substrata. In addition to their mandibles which are used to grasp food materials, New Zealand leptophlebiid nymphs have brush-like maxillae used to sweep loose particles into the mouth. Burrowing mayfly nymphs, including the New Zealand species of *Ichthybotus* ingest fine particles from within the stream bed, whereas others such as *Coloburiscus humeralis* are filter-feeders that capture particles in the water column using their strongly setose forelimbs and mouthparts. *Ameletopsis perscitus* is probably New Zealand's only carnivorous mayfly.

Mayflies are used globally, including in New Zealand, as biological indicators of degraded water quality because many are sensitive to organically enriched water, inorganic pollutants such as those found in mining discharges, and changes in water temperature and flow patterns (Winterbourn 1981; Stark et al. 2001; O'Halloran et al. 2008; Bauernfeind & Soldán 2012). All life stages of mayflies provide important food-web links between basal algal/detrital resources and higher trophic levels including predatory freshwater fish (Sagar & Eldon 1983; McDowall 1990), riverine birds (Pierce 1979, 1986), as well as predatory invertebrates, especially spiders, in adjacent terrestrial ecosystems (Collier et al. 2002; Burdon & Harding 2008; Greenwood & McIntosh 2010).

Systematics of New Zealand Ephemeroptera

Four distinct periods of research activity can be identified in the history of New Zealand mayfly taxonomy and systematics (Pohe 2018). This began with descriptions of the first New Zealand mayflies, *Coloburiscus humeralis* and *Neozephlebia scita* by Walker (1853) (as *Palingenia humeralis* and *Baetis scita*). In the fifty years that followed, a further ten species were added to the fauna by Eaton (1871, 1883–1888, 1899), McLachlan (1873, 1894) and (Hudson 1904). During the 1920s and 1930s Tillyard (1923), Phillips (1930) and Lestage (1935) added another 11 species. In the 1960s Penniket (1962, 1966)

described *Siphlaenigma janae* and *Rallidens mcfarlanei* and between 1971 and 1996 William Peters and David Towns described 17 new species of Leptophlebiidae (Peters 1971; Towns & Peters 1979a, b; Towns 1983; Towns & Peters 1996). In the last 15 years Terry Hitchings and his associates have added 12 further species (Hitchings & Staniczek 2003; Hitchings 2008b, 2009b, 2010; Staniczek & Hitchings 2014; Hitchings & Hitchings 2016) and Winterbourn (2009) added a new genus with a single species. A number of undescribed species are known (T. Hitchings, pers. comm., 2018), and it is likely that others will be found.

In recent decades there has been an increasing amount of molecular research done on aquatic insects, but the number of genetic studies on New Zealand freshwater insects has been relatively low. However, some molecular studies on Odonata (Nolan et al. 2007; Marinov et al. 2016), Trichoptera (Smith & Collier 2001; Smith et al. 2006a), Plecoptera (McCulloch et al. 2009; McCulloch 2010; McCulloch et al. 2010, 2016; Winterbourn et al. 2017), Diptera (Morris 2005; Craig et al. 2012) and Megaloptera (Hogg et al. 2002) have been undertaken. Studies of population genetic structure have also been made on several species of New Zealand Ephemeroptera. Thus, Smith and Collier (2001) and Smith et al. (2006b) worked with *Acanthophlebia cruentata*, Hogg et al. (2002) and Wallace (2013) examined populations of *Coloburiscus humeralis* and Morris (2005) investigated *Nesameletus vulcanus*, *N. ornatus* and *C. humeralis*.

Phylogenetic studies based on morphological criteria have been made on New Zealand Leptophlebiidae by Towns and Peters (1980, 1996) and representatives of all eight New Zealand families have been included in global phylogenetic studies of the order (Ogden et al. 2009, and references within). A molecular study of phylogenetic relationships among Leptophlebiidae of the world by O'Donnell and Jockusch (2008) included representatives of eight New Zealand genera.

Patterns of diversity and distribution

The recorded distribution of New Zealand mayfly species reflect both the country's past geological and climatic history, and the changing availability of present-day habitat. Currently about 40% of the New Zealand mayfly fauna is known to occur throughout the length of the country (Hitchings 2008a), with a high proportion of species seeming to be restricted to forested habitats, at least in the North Island. Of the 60% of mayfly fauna that are not nationally distributed, many appear to be regionally or locally endemic and restricted to island or island-like habitats (i.e. forest fragments, peninsulas or mountain ranges). In some groups current distribution records are patchy, and in yet others where the differentiation of species is difficult, or currently not possible, distribution patterns are unclear (Macfarlane et al. 2010).

Following the last glacial period 14,000 years ago, native forest covered 82% of the land surface of New Zealand below the alpine treeline (Ewers et al. 2006). However, today only 23% of New Zealand has native forest cover (Leathwick et al. 2003). As forest ecosystems substantially moderate water and air

temperatures (Meleason & Quinn 2004) the absence of forest is likely to have a strong influence on the distribution of temperature-sensitive mayflies. Mayflies have a narrow tolerance to changes in environmental conditions, and a particularly low thermal tolerance (Quinn et al. 1994). Impaired water quality resulting from changes in land use (e.g. horticulture, agriculture, forestry and mining) can also have a strong influence on the distributions of mayflies as mentioned above (Quinn & Hickey 1990; Harding & Winterbourn 1995).

At the global scale a considerable amount of literature describes high species richness in the tropics and a decline in richness at higher latitudes (Rohde 1992). Consistent with this, but at a much smaller scale, Towns and Peters (1996) indicated that more mayfly species were known from North Island streams than from similar streams in the South Island. Furthermore, within the North Island more species have been reported from lowland than high-altitude streams. Although this seems to contradict the suggestion that mayflies have low thermal tolerance, most mayflies in northern regions do tend to occur in forests (Quinn et al. 1997) where air and water temperatures are buffered, and flows are often more stable. Interestingly, the recent description of 12 new mayfly species from the South Island (Hitchings & Staniczek 2003; Hitchings 2008b, 2009b, 2010; Staniczek & Hitchings 2014; Hitchings & Hitchings 2016), and the existence of others awaiting description (T. Hitchings, pers. comm., 2018) has closed the perceived latitudinal diversity gap considerably. It indicates that our understanding of mayfly distribution and diversity patterns has been overly influenced in the past by the much greater degree of taxonomic work in the North Island. Towns and Peters (1996) also alluded to imprecise identifications as contributing to the poor understanding of New Zealand mayfly distributions; a limitation they made a giant step towards rectifying with their Fauna of New Zealand publication on Leptophlebiidae (Towns & Peters 1996).

The effects of temperature and climate warming on stream fauna

Body size is a fundamental species trait shaping the structure and function of aquatic communities (Woodward et al. 2005). It has a direct relationship with metabolism, resource use and species abundance, as well as important life history traits such as fecundity and dispersal (Clifford & Boerger 1974; Vannote & Sweeney 1980; Sweeney et al. 1992; White et al. 2007). Water temperature, in turn, is a key physical parameter affecting the growth, metabolism, reproduction, emergence and distribution of aquatic insects (Hynes 1970), and therefore affects life history patterns (Harper & Peckarsky 2006). In New Zealand, water temperature was found to directly control growth, development and emergence patterns of the leptophlebiid mayfly *Deleatidium lillii* (Huryn 1996).

Temperature also has much wider effects on ecosystem resources and functioning. For example, increases in water temperatures can alter the carbon cycle directly, by increasing primary production and ecosystem respiration, while shifting metabolic balance by reducing CO_2 sequestration and resource

quality (Yvon-Durocher et al. 2010). Nevertheless, in some cases bottom-up effects of warming such as these may theoretically be countered by top-down predation (Greig et al. 2012).

Although there is much political and social debate surrounding climate change and global warming, strong data exist which show that climate is changing. Mean annual global surface temperature has risen 0.8°C in the last century (Hansen et al. 2006) and is predicted to increase by 3–5°C over the next century (IPCC 2007). Similarly, New Zealand's annual mean surface temperature increased by 0.9°C between 1908 and 2006 (Mullan et al. 2008) and there has been an increased frequency of extreme weather events, warmer mean annual temperatures, reduced frost frequency, retreating glaciers and snowlines, and reduced alpine snowfall recorded (McGlone & Walker 2011). Many of these changes differ between regions, and likely result from changes to atmospheric circulation patterns (Salinger & Mullan 1999; Clare et al. 2002). How such changes will affect biodiversity is unclear but common predictions listed by McGlone and Walker (2011), are:

- migration of species from current geographical or altitudinal ranges
- alterations in timing of seasonal events (life histories)
- alterations in species interactions due to the above changes
- alterations of trophic interactions, and
- increased success of non-indigenous biota

Climate change is expected to have greater impacts on ecosystems at higher latitudes and altitudes than in the tropics (Schröter et al. 2005; Hansen et al. 2006) and freshwater ecosystems may be particularly vulnerable because much of the freshwater biota is perceived to have limited dispersal ability and aquatic ecosystems are often physically fragmented within a largely terrestrial landscape (Woodward et al. 2010). Furthermore, water availability and water temperature (which is critical to dissolved oxygen supply and organism metabolism and growth) are both climate-dependent.

Although it is not yet fully understood how climate change will impact New Zealand ecosystems, modelling can be used to calculate possible scenarios under simulated warming conditions. For example, based on projections of climate-change effects on New Zealand alpine plant biodiversity, Halloy and Mark (2003) predicted that 40–70% of indigenous alpine plant species would be at risk of extinction if the present 100-year mean temperature increase (0.6°C) were maintained. In addition, they predicted that fragmentation of alpine areas, and a resulting loss of ~80% of existing alpine 'islands', would compound extinction risks, considerably.

With particular respect to aquatic ecosystems in New Zealand, Scarsbrook (2006) found that our coolest rivers had shown a warming trend of 1.6°C between 1989 and 2005, and Ryan and Ryan (2006) concluded that "regardless of the magnitude of climate change in New Zealand there will be impacts on our freshwater organisms and ecosystems." In particular, they indicated that organisms unable to

disperse to cooler areas, or adapt to higher temperatures, will be extirpated from parts of their current ranges. For aquatic insects such as mayflies one prediction is that a major ecological response to global warming will be a seasonal shift in life-cycles as well as shifts in species ranges toward higher altitudes and latitudes (Winterbourn et al. 2008; Daufresne et al. 2009). Hitchings (2009a) also suggested that a warming climate, and resulting changes in aquatic habitats, would likely cause the extinction of a number of specialised alpine mayfly species. Elsewhere, recent studies have shown that range expansions and migrations to colder latitudes have already begun (Yvon-Durocher et al. 2010; Telwala et al. 2013, and references within).

Research aims and thesis outline

My thesis focuses on investigating the patterns of diversity and distribution of the New Zealand mayfly fauna. I also extended this research to explore aspects of the ecology, population structure and body size of selected species, some of which were poorly known. Specifically, the main aims of my research were to:

1). Clarify and extend the known distributions of New Zealand Ephemeroptera, and make an assessment of the distributions of mayflies across latitudinal and altitudinal gradients. I did this by conducting a comprehensive nationwide survey of the three main islands of New Zealand (North, South and Stewart Islands). My survey design incorporated a multi-level stratified sampling plan and standardised collecting methods, and targeted nymphal, subimaginal and imaginal life stages.

2). Combine the distribution data generated from my nationwide survey with historical records of specimens, including those in the Ministry for the Environment's national macroinvertebrate monitoring database, and museum collections.

3). Improve our understanding of the ecological, genetic, and conservation status of two poorly studied species, *Siphlaenigma janae* and *Isothraulus abditus*.

The thesis consists of five data chapters, a synthesis and appendices. Four of the chapters have been published and are presented in their published format, while one chapter has a more traditional thesis format. References cited in the text are provided at the end of each chapter.

Chapter 2 is a published paper presenting a formal checklist of the described New Zealand fauna, annotated with type specimen, distribution and conservation status information. **Publication:** Stephen R. Pohe 2018. An annotated checklist of New Zealand mayflies (Ephemeroptera), 2018. New Zealand Natural Sciences 43: 1–20.

Chapter 3 has also been published and describes the results of an experiment I conducted to determine the best types and combination of lights for attracting flying aquatic insect stages, especially

mayflies. This informed the methods for my nationwide survey. **Publication:** Stephen R. Pohe, Michael J. Winterbourn & Jon S. Harding 2018. Comparison of fluorescent lights with differing spectral properties on catches of adult aquatic and terrestrial insects. New Zealand Entomologist 41: 1–11.

In Chapter 4 (the unpublished chapter) I present the results of a comprehensive analysis of the diversity and distribution patterns of the New Zealand mayfly fauna and examine the influence of latitude and altitude as drivers of mayfly species richness and body size.

Chapters 5 and 6 comprise two published papers presenting aspects of the ecology, distribution, population structure and conservation status of two poorly understood New Zealand mayflies — *Siphlaenigma janae* and *Isothraulus abditus*. **Publication 1:** Stephen R. Pohe, Michael J. Winterbourn, Sharyn J. Goldstien & Jon S. Harding 2018. Distribution, body size, genetic structure and conservation of *Siphlaenigma janae* (Insecta: Ephemeroptera). New Zealand Journal of Zoology 45: 154–170. **Publication 2:** Stephen R. Pohe, Michael J. Winterbourn, Sharyn J. Goldstien, Olivier J.-P. Ball & Jon S. Harding 2019. Distribution, nymphal habitat, genetic structure and conservation of the New Zealand mayfly *Isothraulus abditus* (Insecta: Ephemeroptera) and a description of its subimago. New Zealand Journal of Zoology 46: 13–30.

Chapter 7 is a synthesis that draws together all aspects of my research and provides an overview of how my work has extended our understanding of the mayfly fauna.

Also included at the end of the document are three appendices containing additional data. Appendix 1 provides a detailed list of survey site data, relating to Chapter 4. In Appendix 2 I provide comments on some taxonomic issues and molecular data I generated during the research, but which do not fit within the scope of the presented thesis. Appendix 3 is a published paper based on data collected during the nationwide survey that explores the aquatic invertebrate communities of intermittent streams in the Little Barrier Island Nature Reserve in northern New Zealand.

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Chapter 2 – *Summarising the New Zealand mayfly fauna: an annotated checklist*

"Those living today will either win the race against extinction or lose it, the latter for all time. They will earn either everlasting honour or everlasting contempt."

Prof. Edward O. Wilson, 2006

Images: Olly Ball / Steve Pohe Collection.

Chapter 2 preface

This chapter presents a formal summary of the New Zealand mayfly fauna and has been published as Stephen R. Pohe. 2018. An annotated checklist of New Zealand mayflies (Ephemeroptera), 2018. New Zealand Natural Sciences 43: 1–20. Distribution data presented in this chapter are generated directly from Chapter 4 of this thesis. The journal's style and conventions have been retained but some of the formatting has been adjusted to fit the broader thesis layout.

Technical note: Since the time this manuscript was published Hitchings and Hitchings (2018) have described two additional species, bringing the New Zealand faunal count to 57.

Abstract

The New Zealand mayfly (Ephemeroptera) fauna currently comprises 55 described species belonging to 20 genera in eight families. All species and genera are endemic to New Zealand, as are three families. The purpose of this paper is to draw together the fragmented information of published literature in the form of a checklist of New Zealand Ephemeroptera. The checklist is annotated with species authority and publication details, type specimen information (sex, life stage, type locality, collector, specimen repository), distribution and conservation status data, and previously recognised names, including synonyms. References to all valid species descriptions and previously recognised names are included in the bibliography.

Keywords: New Zealand, mayflies, Ephemeroptera, type localities, type specimens, species distributions, conservation status.

Introduction

The mayflies (Ephemeroptera) are an ancient insect lineage dating back over 300 million years and are believed to be the most primitive group of extant winged insects (Grimaldi & Engel 2005; Bauernfeind & Soldán 2012). They are represented on all continents except Antarctica, but have strong distributional affinities to specific biogeographical regions (Gressitt 1967; Barber-James et al. 2008). Only three families have global distributions, the remaining 39 being fairly evenly distributed between the northern and southern hemispheres. Globally, about 3,600 mayfly species have been described (Barber-James et al. 2013; M. Sartori and H. Barber-James pers. comm., May 2018; P. Suter pers. comm., May 2018), making the Ephemeroptera one of the smaller orders of insects.

The New Zealand mayfly fauna comprises 55 described species in 20 endemic genera and eight families, three of which are also endemic to New Zealand (Ichthybotidae, Rallidentidae, Siphlaenigmatidae). They have close phylogenetic relationships with the cold-adapted mayflies of southern South America and Australia, land masses which like New Zealand, were parts of the ancient

continent of Gondwana (Edmunds 1975; Tsui & Peters 1975; Gibbs 2016). The first mayflies described from New Zealand were *Coloburiscus humeralis* (Walker, 1853) (as *Palingenia humeralis*) and *Neozephlebia scita* (Walker, 1853) (as *Baetis scita*). In the following 50 years, nine valid species were described by Eaton (1871, 1883–1888, 1899) and McLachlan (1873, 1894). Eaton (1899) also provided the first annotated checklist of New Zealand Ephemeroptera (as Ephemeridae), which included 11 species. Subsequently, Hudson (1904) published a popular account of the biology of New Zealand aquatic insects that also included one new mayfly species, and Tillyard (1923) added two more. Phillips (1930) made a comprehensive revision of the New Zealand mayfly fauna, including descriptions of eight new species, but in the following 50 years only four more species were described (Lestage 1935; Penniket 1962, 1966; Peters 1971).

Checklists of the aquatic and water-associated insects of New Zealand, which included mayflies, were published by Wise (1965, 1973) who also produced a synonymic checklist of the smaller hexapod orders that listed 27 mayfly species (Wise 1977). Included amongst a large series of papers on the New Zealand Leptophlebiidae, Towns and Peters (1979a, b) and Towns (1983b) described eight species in seven genera, and eight further species were included in their subsequent monograph (Towns & Peters 1996), which elevated the total number of New Zealand mayflies to 42. More recently, a revision of the Nesameletidae by Hitchings and Staniczek (2003) added three species of *Nesameletus*. Eight species of *Deleatidium* (Hitchings 2008, 2009, 2010; Hitchings & Hitchings 2016) and a species of *Rallidens* (Staniczek & Hitchings 2014) have also been described by these authors. A new genus and species of Leptophlebiidae, *Aupouriella pohei*, was also described by Winterbourn (2009). Other valuable New Zealand mayfly contributions include the distribution maps of specimens held in the Canterbury Museum (Hitchings 2001; Hitchings et al. 2015) and a chapter in Volume 2 of '*The New Zealand Inventory of Biodiversity*' (Gordon 2010), which provides an excellent summary of the New Zealand mayfly fauna and includes a list of 48 species.

The purpose of this paper is to draw together fragmented information from the published literature in the form of a checklist of New Zealand Ephemeroptera. The checklist is annotated with species authority and publication details, type specimen information (sex, life stage, type locality, collector, specimen repository), distribution and conservation status data, and previously recognised names, including synonyms. References to all valid species descriptions, and to previously recognised names, are included in the bibliography.

Format of the checklist

The higher classification (suborder and family) of the New Zealand Ephemeroptera used in this paper is based on "McCafferty's system" presented in Ogden and Whiting (2005, see Figure 2B). However, it is acknowledged that the status of the suborders Pisciforma and Setisura are in question (Ogden et al. 2009) and may be subject to change. The higher classification is presented first, followed by an

annotated checklist of genera and species in each family. Binomial names are listed in alphabetical order by family, genus and, when recognised, subgenus. These are annotated with species authority and publication page number, and with type specimen information if known (sex, life stage, type locality, collector, specimen repository). Specimen data are followed by recognised species distributions and conservation status classifications. Distributions are indicated by the two letter locality codes of Crosby et al. (1998) (Figure 2.1) and North, South, and Offshore Island localities are separated by two solidus symbols (//). Conservation status classifications are sourced directly from the most recent listings for New Zealand freshwater invertebrates (Grainger et al. 2014). Finally, other names by which valid species have been known are given, and it is stated whether these names were by original designation (orig.), synonyms (syn.) or the result of recombination (comb.). Further, notes are also given to indicate that a name was published without a description of the taxon (undesc.), was misspelt (spell.), or was the result of misidentification (misid.). Reference details are provided for each entry.

Documentation of species in the above format enables one to search for any published name, including synonyms, and it provides easy access to relevant publication information including page number, type data, known distributions and conservation status. Distribution data provided for each species are based on records in the literature, records from official New Zealand databases (National Rivers Water Quality Network) and collections (New Zealand Arthropod Collection, Museum of New Zealand, Canterbury Museum, Otago Museum), and data from a nation-wide New Zealand mayfly field survey of adults and nymphs, undertaken by the author during 2013–2016. The combined dataset from all sources currently comprises 73,459 records and forms the basis of an inaugural national mayfly database presently under construction by the author. However, it is by no means exhaustive, as material exists in several national collections that are yet to be assessed, i.e., part of the New Zealand Arthropod Collection, the Auckland Museum Collection and the NIWA Collection.

Status of type material in Canterbury Museum, Museum of New Zealand and New Zealand Arthropod Collection were confirmed by their respective collection curators. However, at the time of publication no response had been received from The Natural History Museum, London, regarding type material held there. In addition, the type repository of *Coloburiscus tonnoiri* Lestage, 1935 is currently unknown but "may be in a museum in Brussels" (Terry Hitchings, Canterbury Museum Research Fellow, pers. comm., 11 April 2018). The following abbreviations are used to indicate repositories of type material listed in the checklist:

BMNH - The Natural History Museum, London, England
CMNZ - Canterbury Museum, Christchurch, New Zealand
NMNZ - Museum of New Zealand/Te Papa Tongarewa, Wellington, New Zealand
NZAC - New Zealand Arthropod Collection, Landcare Research, Auckland, New Zealand

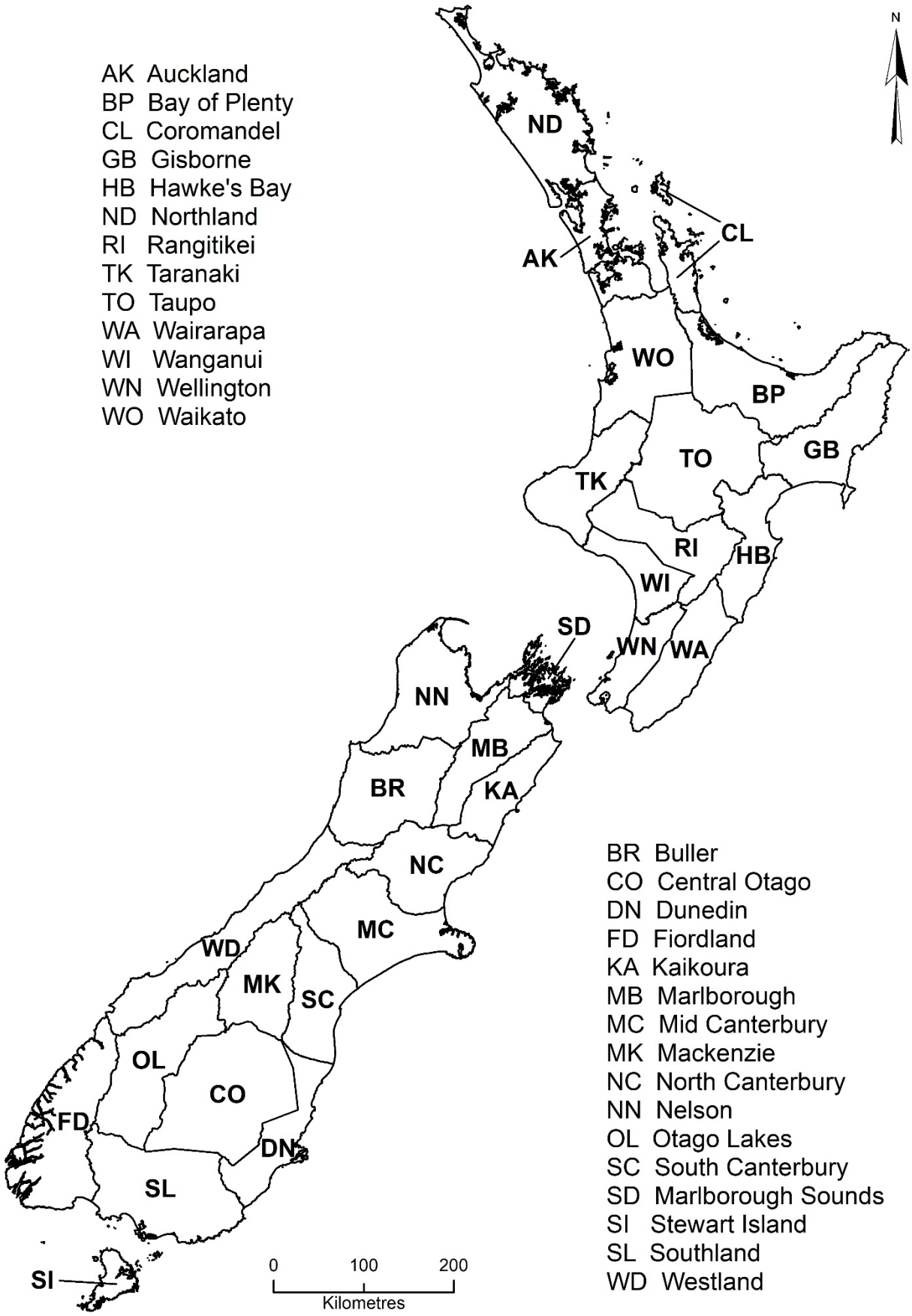

Figure 2.1 Map of New Zealand with area codes used to describe specimen distribution records. For more details, including offshore island area codes, see Crosby et al. (1998).

Higher classification of New Zealand Ephemeroptera

Class: Insecta

Order: Ephemeroptera Hyatt & Arms, 1890: 69

Suborder: Furcatergalia

Family: Ichthybotidae Demoulin, 1957: 336

Family: Leptophlebiidae Banks, 1900: 246 (as Leptophlebini)

Suborder: Pisciforma

Family: Ameletopsidae Edmunds, 1957: 246 (as Ameletopsinae)

Family: Nesameletidae Kluge et al., 1995: 115

Family: Oniscigastridae Lameere, 1917: 62 (as Oniscigastrina)

Family: Rallidentidae Penniket, 1966: 169 (as Rallidentinae)

Family: Siphlaenigmatidae Penniket, 1962: 394

Suborder: Setisura

Family: Coloburiscidae Edmunds in Edmunds et al., 1963: 11 (as Coloburiscinae)

Species checklist of New Zealand Ephemeroptera

AMELETOPSIDAE

***Ameletopsis* Phillips, 1930: 324**

Ameletopsis perscitus (Eaton, 1899): 291, "♀imago, No. 26. Wellington (Hudson)". Holotype: BMNH. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, RI, WN, WA // SD, NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, DN, SL, FD // SI. Conservation status: Not Threatened.

- *Ephemera,* n.s., near *Coloburus* (undesc.) (Hudson 1892: 105)
- *Ameletus perscitus* (orig.) (Eaton 1899: 291)
- *Ameletopsis percitus* (spell.) (Mosely 1932: 6)

COLOBURISCIDAE

***Coloburiscus* Eaton, 1888: 332, 346**

Coloburiscus humeralis (Walker, 1853): 552, "♀imago, New Zealand (Sinclair)". Holotype: BMNH. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, HB, RI, WI, WN, WA // SD, NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, DN, SL, FD // SI. Conservation status: Not Threatened.

- *Palingenia humeralis* (orig.) (Walker 1853: 552)
- *Baetis remota* (syn.) (Walker 1853: 564)
- *Coloburus humeralis* (comb.) (Eaton 1868: 89)
- *Coloburus* [= *Coloburiscus*] *humeralis* (comb.) (Eaton 1888: 332)

Coloburiscus tonnoiri Lestage, 1935: 353 (***nomen dubium***), "♂Imago, Nekotupu[1], New Zealand (Tonnoir)". Holotype: repository unknown. Distribution: AK // – . Conservation status: Taxonomically Indeterminate & Data Deficient.

[1] Likely Nihotupu in the Waitakere Ranges, Auckland (see Craig et al. 2012, page 104).

ICHTHYBOTIDAE

Ichthybotus **Eaton, 1899: 285**

Ichthybotus bicolor Tillyard, 1923: 228, "♂Imago, Maitai River, Nelson (Philpott)". Holotype: Was Cawthron Institute, Nelson, now NZAC. Distribution: – // SD, NN, BR, WD, MB, KA, NC, MC, SC, OL, CO, DN. Conservation status: Not Threatened.

Ichthybotus hudsoni (McLachlan, 1894): 270, "Wellington (Hudson)". No holotype designated. Lectotype: "♂Imago, Wellington, (Hudson)", BMNH. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, HB, RI, WI, WN, WA // – . Conservation status: Not Threatened.

- *Ephemera hudsoni* (orig.) (McLachlan 1894: 270)

LEPTOPHLEBIIDAE

Acanthophlebia **Towns, 1983: 28**

Acanthophlebia cruentata (Hudson, 1904): 33, "Wellington". No holotype designated. Lectotype: "♀ subimago, Campbell's Stream, Karori, Wellington (Hudson)", NMNZ. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, HB, RI, WN, WA // – . Conservation status: Not Threatened.

- *Atalophlebia cruentata* (orig.) (Hudson 1904: 33)
- *Zephlebia (Zephlebia) cruentata* (comb.) (Penniket 1961: 9)

Arachnocolus **Towns & Peters, 1979: 444**

Arachnocolus phillipsi Towns & Peters, 1979: 446, "♂Imago, Cascade Stream, Auckland (Towns)". Holotype: NZAC. Distribution: ND, AK, CL, WO, BP, TK // – . Conservation status: Not Threatened.

- Gen. nov. sp. A (undesc.) (Towns 1976: 46, Table 17; —1978a: 367, 369; —1978b: 411)

Atalophlebioides **Phillips, 1930: 336**

Atalophlebioides cromwelli (Phillips, 1930): 385, "Streams round Wellington". No holotype designated. Lectotype: "♀imago, Hutt River, Wellington (inferred as Phillips)", NMNZ. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, HB, RI, WN // SD, NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, DN, SL, FD. Conservation status: Not Threatened.

- *Deleatidium (Atalophlebioides) cromwelli* (orig.) (Phillips 1930: 385)

***Aupouriella* Winterbourn, 2009: 424**

Aupouriella pohei Winterbourn, 2009: 428, "♂Imago, Whiriwhiri Stream, North Cape (Pohe)". Holotype: CMNZ. Distribution: ND // – . Conservation status: Nationally Critical.

***Austroclima* Towns & Peters, 1979: 213**

Austroclima jollyae Towns & Peters, 1979: 220, "♂Imago, Cascade Stream, Auckland (Towns)". Holotype: NZAC. Distribution: ND, AK, CL, WO, BP, TK, TO, WN // SD, NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, DN, SL, FD // SI. Conservation status: Not Threatened.

- *Atalophlebioides* sp. B (undesc.) (Towns 1978b: 411; —1979: 257)

Austroclima sepia (Phillips, 1930): 383, "Streams round Wellington". No holotype designated. Neotype: "♂Imago, Cascade Stream, Auckland (Towns)", NZAC. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, HB, RI, WI, WN, WA // SD, NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, DN, SL, FD // SI. Conservation status: Not Threatened.

- *Deleatidium (Atalophlebioides) sepia* (orig.) (Phillips 1930: 383)
- *Atalophlebioides sepia* (comb.) (Peters & Edmunds 1964: 238)

***Austronella* Towns & Peters, 1996: 23**

Austronella planulata (Towns, 1983): 17, "♂Imago, Glow-worm Grotto, Waitomo Caves, Waikato (Pugsley)". Holotype: NZAC. Distribution: ND, AK, WO, BP, TK, TO, GB, HB, RI, WN, WA // – . Conservation status: Declining.

- *Zephlebia (Zephlebia) planulata* (orig.) (Towns 1983b: 17)

***Cryophlebia* Towns & Peters, 1979: 230**

Cryophlebia aucklandensis (Peters, 1971): 47, "♂Imago, rocky stream, Bivouac Hill, Mt Eden, Auckland Islands (Gressitt)". Holotype: NZAC. Distribution: AI. Conservation status: Naturally Uncommon.

- *Atalophlebioides aucklandensis* (orig.) (Peters 1971: 47)

***Deleatidium* Eaton, 1899: 288**

Deleatidium (Deleatidium) acerbum Hitchings & Hitchings, 2016: 55, "♂Imago, Camp Creek, Westland (Hitchings & Hitchings)". Holotype: CMNZ. Distribution: – // NN, BR, WD. Conservation status: Not yet assessed.

Deleatidium (Deleatidium) angustum Towns & Peters, 1996: 33, "♂Imago, Cascade Stream, Auckland (Towns)". Holotype: NZAC. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, HB, RI, WN, WA. Also unverified reports from SD, NN, WD, KA, MC, SC, CO, SL. Conservation status: Not Threatened.

- *Deleatidium myzobranchia* (misid.) (Towns 1979: 255–256)
- *Deleatidium* nr *myzobranchia* (undesc.) (Towns 1981: 193–194, 197)
- *Deleatidium* sp. A (undesc.) (Towns 1983b: 41–47; —1985: 233; —1987: 353–356)

Deleatidium (Deleatidium) atricolor Hitchings, 2009: 40, "♂Imago, Edwards River, St James Range (Hitchings)". Holotype: CMNZ. Distribution: – // BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, SL, FD. Conservation status: Not Threatened.

Deleatidium (Deleatidium) autumnale Phillips, 1930: 371, "R. Waikanae and R. Hutt, Wellington district". No holotype designated. Lectotype: "♂Imago, Hutt River, Wellington (Phillips)", CMNZ. Distribution: ND, WO, BP, TK, TO, GB, HB, RI, WN, WA // SD, NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, DN, SL, FD. Conservation status: Not Threatened.

- *Deleatidium autumnale* (orig.) (Phillips 1930: 371)

Deleatidium (Deleatidium) branchiola Hitchings, 2009: 45, "♂Imago, Mataura River tributary, Hector Mts (Hitchings)". Holotype: CMNZ. Distribution: – // OL, CO. Conservation status: Naturally Uncommon.

Deleatidium (Deleatidium) cerinum Phillips, 1930: 382, "R. Hutt, Wellington district". No holotype designated. Lectotype: "♂Imago, Hutt River, Wellington (Phillips)", CMNZ. Distribution: ND, AK, WO, BP, TK, TO, GB, RI, WN, WA // SD, NN, BR, WD, MB, NC, MC, SC, MK, OL, CO, DN, SL, FD // SI. Conservation status: Not Threatened.

- *Deleatidium cerinum* (orig.) (Phillips 1930: 382)

Deleatidium (Deleatidium) fumosum Phillips, 1930: 372, "Streams round Wellington". No holotype designated. Neotype: "♂Imago, Hutt River at Kaitoke, Wellington (Towns)", NZAC. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, RI, WN, WA // SD, NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, DN, SL, FD // SI. Conservation status: Not Threatened.

- *Deleatidium fumosum* (orig.) (Phillips 1930: 372)
- *Deleatidium* sp. C (undesc.) (Towns 1979: 257; —1981: 194; —1983a: 40–47; —1985: 233; —1987: 353)

Deleatidium (Deleatidium) kawatiri Hitchings & Hitchings, 2016: 61, "♂Imago, Little Ten Mile Creek, Buller (Hitchings)". Holotype: CMNZ. Distribution: – // NN, BR. Conservation status: Not yet assessed.

Deleatidium (Deleatidium) kiwa Hitchings, 2010: 33, "♂Imago, Borland Burn, South Branch tributary, Fiordland (Ward & Ward)". Holotype: CMNZ. Distribution: – // SL, FD. Also unverified report from SI. Conservation status: Naturally Uncommon.

Deleatidium* (*Deleatidium) lillii Eaton, 1899: 289, "Dunedin (Lillie) and Wellington (Hudson, No. 46)". Holotype missing. Lectotype: "♂Imago, No. 46. Wellington (Hudson)", BMNH. Distribution: ND, AK, CL, WO, BP, TK, TO, RI, WN, WA // SD, NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, DN, SL, FD // SI. Conservation status: Not Threatened.

- *Atalophlebia scita* (misid. & incorrectly attributed to Walker) (Lillie 1898: 167)
- *Deleatidium lillii* (orig.) (Eaton 1899: 289)
- *Deleatidium (D.) lillii* (incorrectly attributed to Walker) (Phillips 1930: 336, 368)
- *Deleatidium lilli* (spell. & incorrectly attributed to Walker) (Mosely 1932: 10)
- *Deleatidium lillii* (comb.) (Ulmer 1938: 105)

Deleatidium (Deleatidium) magnum Towns & Peters, 1996: 40, "♂Imago, Whakapapaiti Stream, Mt Ruapehu (Crawford)". Holotype: NZAC. Distribution: TK, TO. Also unverified reports from NN, NC, MC, SC, MK, CO. Conservation status: Naturally Uncommon.

Deleatidium (Deleatidium) myzobranchia Phillips, 1930: 373, "Hawkes Bay, Wellington, Nelson and Canterbury provincial districts". No holotype designated. Lectotype: "♂Imago, Ngaio, Wellington (Phillips)", CMNZ. Distribution: ND, AK, CL, WO, BP, TK, TO, RI, WN, WA // SD, NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, DN, SL, FD // SI. Conservation status: Not Threatened.

- *Deleatidium myzobranchia* (orig.) (Phillips 1930: 373)
- *Deleatidium* sp. E (undesc.) (Towns 1979: 257)

Deleatidium (Deleatidium) townsi Hitchings, 2009: 36, "♂Imago, Glentui River, Mt Thomas Forest (Ward & Ward)". Holotype: CMNZ. Distribution: – // SD, NN, BR, WD, MB, KA, NC, MC, SC, MK. Conservation status: Not Threatened.

Deleatidium (Deleatidium) vernale Phillips, 1930: 360, "Tributary of the Kaiwarra[2] Stream at Ngaio and Khandallah near Wellington". No holotype designated. Lectotype: "♂Imago, Ngaio, Wellington (Phillips)", CMNZ. Distribution: ND, CL, TO, GB, RI, WN, WA // SD, NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, DN, SL, FD. Conservation status: Not Threatened.

- *Deleatidium vernale* (orig.) (Phillips 1930: 360)

[2] Kaiwharawhara Stream, Wellington.

Deleatidium (Deleatidium) wardorum Hitchings, 2010: 28, "♂Imago, Waterfall Creek, Ashley (Hitchings)". Holotype: CMNZ. Distribution: – // SD, NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO. Conservation status: Not Threatened.

Deleatidium (Penniketellum[3]) cornutum Towns & Peters, 1996: 47, "♂Imago, stream at base of Tasman Glacier (Towns & Towns)". Holotype: NZAC. Distribution: – // BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, DN, FD. Conservation status: Not Threatened.

Deleatidium (Penniketellum[3]) insolitum (Towns & Peters, 1979): 451, "♂Imago, Edwards Valley, nr Arthur's Pass, North Canterbury (Jackson)". Holotype: NZAC. Distribution: – // NC, MK. Conservation status: Nationally Endangered.

- *Penniketellus insolitus* (orig.) (Towns & Peters 1979a: 451)

Deleatidium (Penniketellum[3]) patricki Hitchings, 2008: 37, "♂Imago, Flush Stream, Mt St. Bathans, Central Otago (Patrick & Edwards)". Holotype: CMNZ. Distribution: – // NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, FD. Conservation status: Not Threatened.

[3] I here follow the nomenclature of Towns and Peters (1996) but acknowledge the spelling surrounding the gender may be incorrect. The original designation "*Penniketellus*" of Towns and Peters (1979a) may be the valid designation.

***Isothraulus* Towns & Peters, 1979: 439**

Isothraulus abditus Towns & Peters, 1979: 442, "♂Imago, Small tributary of Waitakere R. nr Anderson's Track, Auckland (Black)". Holotype: NZAC. Distribution: ND, AK, CL, WO, BP, TK, TO, RI // – . Conservation status: Declining.

- *Zephlebia* sp. A (undesc.) (Towns 1978b): 410

***Mauiulus* Towns & Peters, 1979: 224**

Mauiulus aquilus Towns & Peters, 1996: 51, "♂Imago, Rangitukia Stream, Waikato (Summerhays)". Holotype: NZAC. Distribution: ND, CL, WO, TO, WA // – . Conservation status: Naturally Uncommon.

Mauiulus luma Towns & Peters, 1979: 226, "♂Imago, Cascade Stream, Auckland (Towns)". Holotype: NZAC. Distribution: ND, AK, WO, BP, TK, TO, GB, RI, WN // NN, BR, WD, MB. Conservation status: Not Threatened.

- *Atalophlebiodes* sp. A (undesc.) (Towns 1978a: 367, 369; —1978b: 411; —1979: 256)

***Neozephlebia* Penniket, 1961: 9**

Neozephlebia scita (Walker, 1853): 570, “New Zealand”. No holotype designated. Lectotype: one of the original two specimens, “♂Imago lacking head, New Zealand (Sinclair)”, BMNH. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, RI, WN, WA // SD, NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, DN, SL, FD // SI. Conservation status: Not Threatened.

- *Baetis scita* (orig.) (Walker 1853: 570)
- *Leptophlebia nodularis* (syn.) (Eaton 1871: 81)
- *Leptophlebia scita* (comb.) (Eaton 1871: 81)
- *Atalophlebia nodularis* (syn.) (Eaton 1884: 89; —1899: 288; Lillie 1898: 168; Hutton 1898: 216; Hudson 1904: 34; Phillips 1930: 352; Mosely 1932: 8; Kimmins 1960: 295; McLean 1967: 99–104)
- *Atalophlebia scita* (comb.) (Eaton 1884: 90; —1899: 288; Hutton 1898: 216; Hudson 1904: 34; Kimmins 1960: 295)
- *Zephlebia (Neozephlebia) nodularis* (syn.) (Penniket 1961: 9; Winterbourn & Towns 1981: 18)
- *Zephlebia (Neozephlebia) scita* (comb.) (Penniket 1961: 9; Winterbourn & Towns 1981: 18)
- *Zephlebia scita* (comb.) (Cadwallader 1975a: 12, 16, 22; —1975b: 16, 20–21; —1975c: 304–308; Michaelis 1977: 366)
- *Zephlebia scita/nodularis* (syn.) (Towns 1978a: 367)
- *Zephlebia nodularis* (syn.) (Towns 1978b: 410; —1979: 255)

***Tepakia* Towns & Peters, 1996: 54**

Tepakia caligata Towns & Peters, 1996: 55, “♂Imago, Waikoha Stream, Waikato (Summerhays)”. Holotype: NZAC. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, WN, WA // – . Conservation status: Not Threatened.

- "a second species of *Isothraulus*" (undesc.) (Towns & Peters 1979a: 444)

***Zephlebia* Penniket, 1961: 8**

Zephlebia borealis (Phillips, 1930): 356, “Tanekaha, west of Hikurangi, North Auckland”. No holotype designated. Lectotype: “slides of nymphal legs and gills, designated from paratype with no locality data (Phillips)”, BMNH. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, RI, WN // – . Conservation status: Not Threatened.

- *Atalophlebia* ? n. sp. [also provisionally as *A. borealis*] (orig.) (Phillips 1930: 356)
- *Zephlebia (Zephlebia) borealis* (comb.) (Penniket 1961: 9)
- *Zephlebia (Zephlebia)* sp. (cf. *borealis*) (comb.) (Landa et al. 1980: 171)
- *Zephlebia (Terama) borealis* (comb.) (Towns 1983b: 19)

Zephlebia dentata (Eaton, 1871): 80, "New Zealand". No holotype designated. Lectotype: "♂imago, designated by Kimmins (1960) with locality as New Zealand (collector unknown)", BMNH. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, RI, WI, WN, WA. Also recorded from the upper South Island but may be 'in error'. Conservation status: Not Threatened.

- *Leptophlebia dentata* (orig.) (Eaton 1871: 80)
- *Atalophlebia dentata* (comb.) (Eaton 1884: 88; —1899: 287; Hutton 1898: 215; Hudson 1904: 31; Phillips 1930: 344; Mosely 1932: 7; Kimmins 1960: 295)
- *Zephlebia (Zephlebia) dentata* (comb.) (Penniket 1961: 9)

Zephlebia inconspicua Towns, 1983: 12, "♂imago, Cascade Stream, Auckland (Towns)". Holotype: NZAC. Distribution: ND, AK, CL, WO, BP, TO // – . Conservation status: Not Threatened.

- *Zephlebia* n.sp. B (undesc.) (Towns 1978a: 367)
- *Zephlebia* sp. B (undesc.) (Towns 1976: 44; —Towns 1978a: 369; —1978b: 410)
- *Zephlebia (Zephlebia) inconspicua* (orig.) (Towns 1983b: 12)

Zephlebia nebulosa Towns & Peters, 1996: 63, "♂imago, Rangitukia Stream, Waikato (Summerhays)". Holotype: NZAC. Distribution: ND, AK, CL, WO, BP, TK, TO // – . Also unverified reports from BR and SD. Conservation status: Naturally Uncommon.

- *Zephlebia* sp. A (undesc.) (Towns 1987: 352–353)

Zephlebia pirongia Towns & Peters, 1996: 65, "♂imago, Rangitukia Stream, Waikato (Summerhays)". Holotype: NZAC. Distribution: WO // – . Conservation status: Not Threatened.

Zephlebia spectabilis Towns, 1983: 14, "♂imago, Waitakere River, Auckland (Black)". Holotype: NZAC. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, RI, WN, WA // SD, NN, BR, WD, MB, KA, MC, SC, OL, CO, DN, SL, FD // SI. Conservation status: Not Threatened.

- *Zephlebia (Zephlebia)* sp. (undesc.) (Towns 1979: 256; —1981: 194)
- *Zephlebia (Zephlebia) spectabilis* (orig.) (Towns 1983b: 14)

Zephlebia tuberculata Towns & Peters, 1996: 68, "♂imago, Rangitukia Stream, Waikato (Summerhays)". Holotype: NZAC. Distribution: ND, AK, CL, WO, BP, TK, GB, RI // – . Conservation status: Data Deficient.

Zephlebia versicolor (Eaton, 1899): 286, "Wellington (Hudson)". No holotype designated. Lectotype: "♂ imago, Wellington (Hudson)", BMNH. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, RI, WN, WA // SD, NN, BR, MB. Conservation status: Not Threatened.

- *Atalophlebia versicolor* (orig.) (Eaton 1899: 286; Hudson 1904: 30; Phillips 1930: 339; Mosely 1932: 7; Kimmins 1960: 296; Stout 1969: 491; —1973: 244; —1975: 442)
- *Zephlebia (Zephlebia) versicolor* (comb.) (Penniket 1961: 8)

NESAMELETIDAE

Nesameletus **Tillyard, 1933: 11**

Nesameletus austrinus Hitchings & Staniczek, 2003: 24, "♂Imago, Camp Stream, Craigieburn Range (Hitchings)". Holotype: CMNZ (unavailable for confirmation at time of publication). Distribution: – // NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, SL, FD. Conservation status: Not Threatened.

- *Nesameletus* sp. A (undesc.) (Winterbourn 2003: 41–50)

Nesameletus flavitinctus (Tillyard, 1923): 226, "♀imago, Waihi Stream, near Tokaanu (Tillyard)". Holotype: Was Cawthron Institute, Nelson, now NZAC. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, HB, RI, WN // SD, NN, BR, WD, MB, KA, NC, MC, SC, OL, CO, DN. Conservation status: Not Threatened.

- *Ameletus flavitinctus* (orig.) (Tillyard 1923: 226)

Nesameletus murihiku Hitchings & Staniczek, 2003: 22, "♂Imago, Mill Creek, Stewart Island (Hitchings)". Holotype: CMNZ. Distribution: – // OL, CO, SL, FD // SI. Conservation status: Naturally Uncommon.

Nesameletus ornatus (Eaton, 1883): plate XIX, "Christchurch (Wakefield)". Holotype missing. Neotype: "♀imago, Wellington (Hudson, No. 42)", BMNH. Distribution: ND, AK, CL, WO, BP, TK, TO, GB, HB, RI, WN, WA // SD, NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, DN, SL, FD. Conservation status: Not Threatened.

- *Chirotonetes* (?) *ornatus* (orig.) (Eaton 1883: plate XIX; —1885: 208; —1888: 321)
- *Ameletus ornatus* (comb.) (Eaton 1899: 291, plate X)

Nesameletus vulcanus Hitchings & Staniczek, 2003: 26, "♂Imago, Narbey Stream, Banks Peninsula (Hitchings)". Holotype: CMNZ. Distribution: – // MC. Conservation status: Nationally Vulnerable.

ONISCIGASTRIDAE

***Oniscigaster* McLachlan, 1873: 109**

Oniscigaster distans Eaton, 1899: 293, "Wainui-o-mata River, Wellington (Hudson, No. 34 and 34b)". No holotype designated. Lectotype: "♀imago, Wainuiomata R., N.Z. (Hudson, No. 34)", BMNH. Distribution: TK, TO, GB, RI, WN // SD, NN, BR, WD, MB, KA, NC, MC, SC, MK, OL, CO, DN, SL, FD // SI. Conservation status: Not Threatened.

Oniscigaster intermedius Eaton, 1899: 292 (***nomen dubium***), "♀imago, M'Arthur[7], Nelson, 3600 feet (Hudson, No. 34a)". Holotype: BMNH. Distribution: – // NN. Conservation status: Data Deficient.

[7] Mount Arthur, Nelson.

Oniscigaster wakefieldi McLachlan, 1873: 110, "Christchurch, N.Z.". No holotype designated. Lectotype: "♀imago, No. 20. Christchurch, N.Z. (Wakefield)", BMNH. Distribution: ND, WO, TK, TO, GB, WN, WA // SD, NN, BR, WD, MB, KA, NC, OL, CO, DN, SL. Conservation status: Declining.

RALLIDENTIDAE

***Rallidens* Penniket, 1966: 164**

Rallidens mcfarlanei Penniket, 1966: 164, "♂Imago, Waipoua, North Auckland (Penniket)". Holotype: CMNZ. Distribution: ND, AK, CL, WO, BP, TK, TO, RI, WA // – . Also unverified reports from BR. Conservation status: Not Threatened.

Rallidens platydontis Staniczek & Hitchings, 2014: 2, "♂Imago, Otaio River trib., The Hunters Hills (Morris)". Holotype: CMNZ. Distribution: – // NN, MB, NC, SC, OL, DN, SL. Conservation status: Declining.

SIPHLAENIGMATIDAE

***Siphlaenigma* Penniket, 1962: 389**

Siphlaenigma janae Penniket, 1962: 390, "♂Imago, Baxter's Creek, Stillwater, Westland (Penniket)". Holotype: CMNZ. Distribution: ND, AK, CL, BP, TK, TO, GB, RI // BR. Conservation status: Nationally Vulnerable.

- *Siphlaenigma edmundsi* (syn.) (Lugo-Ortiz & McCafferty 1998: 210)

Concluding remarks

Fifty-five New Zealand mayflies have been described, two of which are presently best considered *nomina dubia* (*Coloburiscus tonnoiri* Lestage, 1935 and *Oniscigaster intermedius* Eaton, 1899). The fauna comprises eight families, including three endemic to New Zealand (Ichthybotidae, Rallidentidae, Siphlaenigmatidae), and four shared with Southern Hemisphere continents of Gondwanan origin. The New Zealand species have been described over a 165 year period and can be categorised into five distinct eras based on the time of their description: pre 1905 (12 species), 1920s & 1930s (11 species), 1960s & 1970s (8 species), 1980s and 1990s (11 species) and post 2000 (13 species). Each era is characterised by the activity of a small number of passionate taxonomists who collectively described 0.2–0.7 species per year (Figure 2.2). In the most recent period (the last 18 years) Mr Terry Hitchings, an honorary research fellow at Canterbury Museum and his co-authors, have described 13 new species and been almost solely responsible for advancing mayfly taxonomy in New Zealand.

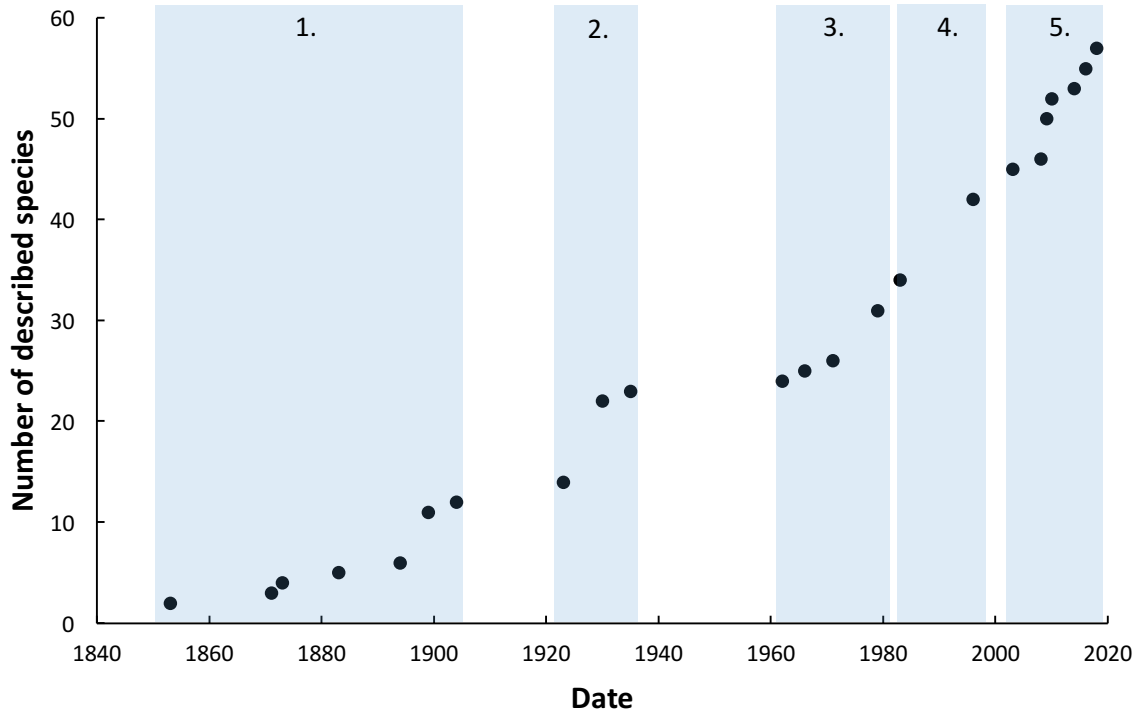

Figure 2.2 Cumulative frequency of New Zealand mayfly species described over time. Pale blue bars indicate the five eras whose primary contributors are: 1. F. Walker, A. Eaton, R. McLachlan & G. Hudson; 2. R. Tillyard & J. Phillips; 3. J. Penniket & W. Peters; 4. W. Peters & D. Towns; 5. T. Hitchings.

The sporadic nature and low rate of New Zealand mayfly descriptions is not a reflection of the actual biodiversity present, but is more likely attributable to the lack of value (and financial support) placed on taxonomic work by New Zealand policy makers and biodiversity managers. A lack of support for taxonomic work is not limited to the Ephemeroptera by any means and has been expressed more broadly by New Zealand entomologists (Lester et al. 2014) and aquatic scientists (Collier et al. 2016), as well as by taxonomists in other parts of the world (see Winterbourn 2014). A general absence of

specialist aquatic insect taxonomists in New Zealand, and particularly at New Zealand universities, has meant that graduates with taxonomic skills are not being produced, which does not bode well for the future of fundamental taxonomic studies of insects, including aquatic taxa, in this country.

That such studies are needed is well illustrated by the mayflies, as a number of undescribed species, mainly in the genera *Deleatidium*, *Nesameletus* and *Zephlebia* are known to be present in both the North and South islands, and preliminary molecular analyses of some described New Zealand species indicate the possible presence of cryptic species in these and other genera (author's unpublished data). Taxonomic studies combining morphological and molecular data are therefore needed to better understand the diversity of the New Zealand mayfly fauna.

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Chapter 3 – *What fluorescent lights are best to attract mayflies to light traps for biodiversity surveys?*

"Never lose your sense of wonder".

Prof. Michael Winterbourn, 2006.

Mangere Stream in Pukenui Forest, Northland. One of the study sites used in the light trapping experiment.

Chapter 3 preface

This chapter describes a study used to establish the best light-trapping approach to collect aquatic specimens for biodiversity surveys. It has been published as Stephen R. Pohe, Michael J. Winterbourn and Jon S. Harding. 2018. Comparison of fluorescent lights with differing spectral properties on catches of adult aquatic and terrestrial insects. New Zealand Entomologist 41: 1–11. https://doi.org/10.1080/00779962.2017.1398704. The journal's style and conventions have been retained but the format of tables and figures have been adjusted to fit the broader thesis layout. I undertook the data collection and analyses, laboratory work and wrote the draft manuscript. My co-author Prof. Harding provided advice on study design and commented on the final draft of the manuscript. Prof. Winterbourn provided assistance with manuscript writing and commented on drafts of the manuscript. Training and assistance for statistical modelling was provided by Dr Helen Warburton and two statistical consultants.

Abstract

The effectiveness of four different fluorescent light sources, used to attract adult aquatic insects (Ephemeroptera, Plecoptera, Trichoptera) and co-occurring terrestrial insects (Lepidoptera, Coleoptera, Diptera), was investigated. Blacklight (BL), blacklight-blue (BLB), cold white light (CW) and a blacklight/cold white light (BL/CW) combination were compared in a field trial with a fully-crossed factorial design. Each light treatment was also assessed at two levels of power (intensity): 16- and 32-watts. All light treatments attracted insects belonging to the six orders, but on average BLB and BL caught nearly three times more aquatic insects than CW light, and about 1.5 times more terrestrial insects. The combination lights generally attracted intermediate catch numbers. Overall, BLB was most effective for attracting Ephemeroptera and Trichoptera, whereas blacklight attracted the most terrestrial insects, particularly Lepidoptera. Doubling the number of lights generally resulted in larger catches, but not significantly so ($P > 0.05$). We recommend that BLB lights be used in studies of adult aquatic insects because they are as effective as regular BL and appear to reduce the likelihood of attracting non-target terrestrial species; a favourable outcome with regard to faunal conservation and, more practically, sample sorting. Our results suggest that, for studies in remote locations, 16-watts of light should be sufficient to obtain reasonable catches and, importantly, reduce the need to carry additional equipment, including heavy batteries.

Introduction

Light trapping is an important sampling method used by entomologists as it exploits the positive phototactic response of many nocturnal, flying insects. Different insect groups use an array of spectral wavelengths for visual perception (Tovée 1995; Briscoe & Chittka 2001), although it has been suggested

that light in the ultraviolet spectrum provides insects with the best indication of a clear flight path through vegetation (Blomberg et al. 1976). Whether insects are physiologically attracted to light, or merely disoriented and diverted from their original flight trajectories, is not clear. Nevertheless, trapping using artificial lighting is used globally to collect terrestrial insects for taxonomic and ecological research (e.g. Summerville 2014; Wolbert et al. 2014; English et al. 2017), as well as for surveillance and control of pest insects in a number of applied disciplines including biosecurity, agriculture and disease prevention (see Shimoda & Honda 2013).

Aquatic insects are no exception and their phototaxic behaviour has been used to collect adult specimens in New Zealand for both taxonomic (e.g. Ward 1995, 1997, 1998; Ward & Henderson 2004; Winterbourn 2009) and ecological studies (e.g. Collier et al. 1997; Collier & Smith 1998; Smith et al. 2002; Pohe 2008; Winterbourn & Pohe 2017; Winterbourn et al. 2017). To understand the ecology and life history of the ephemeropteran *Coloburiscus humeralis* (Walker, 1853), Tan (1961) used a Tilley lamp placed on a white sheet to collect specimens in the Waitakere Ranges, Auckland. In studies assessing flight activity and flight periodicity, McLean (1967a) and Norrie (1969) used 125-watt mercury vapour lamps to attract adult aquatic insects. Norrie's data were subsequently used in a comparative study of aquatic insect flight periodicity by Ward et al. (1996) who also presented new data collected with a 160-watt mercury vapour lamp and an 8-watt blacklight-blue fluorescent light. A 6-watt fluorescent blacklight was used to trap caddisflies in several ecological studies by Collier, Smith and colleagues (Collier et al. 1997; Collier & Smith 1998; Collier et al. 2000; Smith et al. 2002). Even submerging lights into freshwater habitats has been used in the United States to collect juvenile aquatic insects (see Carlson 1972, and references therein). However, although light trapping is frequently used, there has been little evaluation of the comparative effectiveness of different light sources for attracting aquatic insects.

The advantages of light trapping for aquatic insects include specimens being in generally better condition for identification purposes, the adult specimens being trapped are more likely to be identifiable to species level than juvenile forms, and that lights draw insects from a range of microhabitats, which are often difficult to sample with other methods. The lights used can range from low-intensity fluorescent tubes (e.g. 6-watts) run by batteries, to very high-intensity mercury vapour lamps (e.g. 200-watts) run by a generator. However, the larger and more powerful the equipment, the less portable the set-up becomes, which is often a disadvantage, particularly in remote localities. Furthermore, ecological studies are usually designed with multiple sampling sites, which increases the logistical nature of the study, and the equipment required.

Numerous trapping studies of terrestrial insects have shown that the nature and intensity of the light source influences the effectiveness of a light trap (Heath 1966; Belton & Pucat 1967; Hill 1977; Walker & Galbreath 1979; Venter & Hermanides 2006; Pawson et al. 2009) and thus by inference we

expect it would also affect the likelihood that aquatic species be captured. We recognise that all insect traps are selective to some degree and that catches are influenced by weather, air temperature, season, time of night, the nature of the set up and the general behavioural characteristics of the insects (Hardwick 1968; Baker & Sadovy 1978; Andersen 1979; Bishop et al. 2000; García-López et al. 2011). Furthermore, some species are not attracted to lights at all, or are day-active (McLean 1967b; Patrick 1992; Smith et al. 2002; Henderson & Ward 2007), thus rendering light trapping ineffective. Nevertheless, it is desirable that the characteristics of lights used optimise the likelihood that targeted taxa be obtained. Therefore, the primary aim of our study was to investigate the effectiveness of different types and intensities of portable, low-powered fluorescent lights for trapping adult aquatic insects. The opportunity was also taken to examine catches of co-occurring terrestrial insects.

Methods

Study sites and experimental design

The experimental study was carried out from 19–26 October 2013 in Pukenui Forest (35° 42.6′ S; 174° 15.6′ E), a diverse podocarp–broadleaf forest in Whangarei, Northland (Manning 2001). Traps consisted of white pans (50 x 36 x 5 cm) half-filled with water and 30 mL of biodegradable Surfax® detergent added to reduce water surface tension and so enhance retention of trapped insects. Fluorescent tubes were positioned inside a light housing that was placed immediately above the pan (Figure 3.1).

Figure 3.1 A pan trap set with four fluorescent blacklight-blue light tubes. The number and types of tube were interchanged to achieve the desired spectra and intensity. The waterproof box to the left of the tray contains the 12-volt power supply and 24 hour/7 day timing module.

The tubes were powered by two 12-volt batteries, run in parallel, and operated for four hours starting at sunset. This period of operation was in accordance with the findings of Norrie (1969) and Ward et al. (1996) who suggested that flight activity of New Zealand mayflies and caddisflies was highest at that time. The study was carried out during an eight-day period of fine weather. Air temperature was measured at sunset, 1.5 m above each stream using Hobo® pendant data loggers (Model UA-002-64, Onset Computer Corporation), and ranged from 12.8–16.0 °C (mean 13.7, SEM 0.2 °C).

Sites were located alongside low altitude stony streams with complete forest canopies (95–97% cover), in headwater catchments of the Mangere River (3 sites) and the Hātea River (2 sites) (Table 3.1). Stream sites were separated by 0.4–2.2 km ensuring that light from any one site was not visible at another. Benthic faunas of the streams were similar in species composition, although there were differences in the relative abundances of some species (benthic data not presented).

Table 3.1 Sampling site locations, altitude above sea level and mean stream wetted widths. Stream names marked with an asterisk (*) are informal designations. Coordinates are in New Zealand Geodetic Datum 2000 (version 20160701).

Stream	Latitude (S)	Longitude (E)	Altitude (m)	Width (m)
Pukenui Stream*	35° 42.114′	174° 15.988′	185	2.9
Kauri Stream*	35° 42.275′	174° 15.698′	210	2.6
Ark Stream*	35° 42.540′	174° 14.917′	135	1.5
Mangere Stream	35° 42.734′	174° 14.766′	130	3.7
Pekapeka Stream*	35° 42.847′	174° 15.045′	135	2.7

A fully-crossed factorial design was used to compare the abundance of insects attracted to four contrasting fluorescent light tubes: blacklight (BL) made by CH Lighting®, blacklight-blue (BLB) made by Philips Lighting®, cold white light (CW) made by General Electric® and a combination treatment containing blacklight and cold white light (BL/CW) (all tubes model F8T5; 300 x 16 mm). In addition, two power levels of each light treatment were compared, 16- and 32-watts, obtained using either two or four 8-watt tubes (see Figure 3.1). Thus, every night at the five stream sites, five different light treatments were tested, and on each consecutive night the light treatments were changed. Treatment–Site–Date permutations were determined randomly. By the end of the eight nights of sampling, all four light treatments, at both power levels, had been tested once at each of the five stream sites. A control treatment consisting of an identical, but non-operational light trap was established 50–65 m downstream of each light treatment (n = 40 in total), which in all cases was out of sight of the upstream operational light.

Light spectra

Spectral irradiance, more commonly referred to as intensity, is the light energy received at a surface for a particular wavelength, and was quantified for each light type. Testing was done in a temperature-

controlled laboratory darkroom, set at 16 °C to simulate field temperature on a mild evening (as temperature affects light efficiency; see Discussion). A fibre-coupled optical spectrometer (Model USB2000+, Ocean Optics), calibrated with a LS-1-CAL light source and CC-3 cosine corrector, was used to measure absolute spectral irradiance (μW/cm^2/nm). During testing, the spectrometer was positioned 15 cm above the light source and data were captured in OceanView spectroscopy software version 1.5.2.

Collection and identification of catches

Traps were emptied each morning and catches sorted into the aquatic orders Ephemeroptera, Plecoptera and Trichoptera, and the predominantly terrestrial orders Lepidoptera, Coleoptera and Diptera. The trichopteran family Hydroptilidae was excluded from counts due to the high numbers of individuals captured, and the limited time available for processing them in the field. Subsequent reference to Trichoptera is therefore exclusive of Hydroptilidae. Counts of all members of each order were made in the field, after which those belonging to the terrestrial orders were discarded. Ephemeroptera, Plecoptera and Trichoptera were preserved in 95% alcohol along with other specimens of uncertain identity, which were later identified in the laboratory under a Nikon 10–63x stereomicroscope (Model SMZ800). Mayflies were identified to species level using the keys of Phillips (1930) and Towns and Peters (1996), and original taxonomic descriptions.

Data analysis

Total numbers of insects belonging to each order, and the total numbers of aquatic and terrestrial orders combined as groups, were determined for each light treatment. Species richness of Ephemeroptera, and abundance of the four numerically dominant ephemeropteran species, were also determined. The effects of light type and light intensity on abundance of each insect group, and on species richness of Ephemeroptera, were examined using Poisson Generalised Linear Mixed-effects Models (GLMMs) with a canonical log-link function.

Poisson GLMMs were most appropriate to model the count data and were run with the package '*lme4*' (Bates et al. 2015) in 'R' version 3.3.1 (R Core Team 2016). Response variables (insect abundance and species richness) were modelled as a function of the fixed variables 'light type' and 'light intensity', and their interaction. The fixed effect of 'air temperature at sunset' was included in all models because air temperature is known to influence insect flight activity. Random effects were included in the model to account for the effects of different 'sites' and 'sampling dates'. To account for over-dispersion we included an 'observation-level' random effect in each model (Harrison 2014). To determine the best model for predicting insect abundance we used likelihood-ratio tests and Akaike's Information Criterion (AIC) (Akaike 1973) to see whether predictor variables were explaining a significant proportion of variation in the response. We also evaluated model fit based on the GLMMs marginal R^2 (variance explained by fixed effects alone) and conditional R^2 (variance explained by fixed and random effects

together) approximations of Nakagawa and Schielzeth (2013), calculated with the 'piecewiseSEM' package (Lefcheck 2016). Because likelihood-ratio tests and AIC statistics showed no interaction between light intensity and light type, and no effect of light intensity on insect abundance, abundance data from both intensity levels were combined to assess the effects of light type. Posthoc Tukey's pairwise HSD tests were used to determine significant abundance differences between light types. Results were considered to be significant at alpha < 0.05.

Results

Spectral characteristics of tubes

Blacklight tubes emitted mostly ultraviolet light (peak wavelength 369 nm) with absolute spectral irradiance peaks of c. 185 µW/cm^2/nm (32-watts) and c. 100 µW/cm^2/nm (16-watts), but also produced lesser peaks at 404, 435 and 546 nm (Figure 3.2). Blacklight-blue tubes emitted only ultraviolet light, with the 369 nm peak, but only emitted about half the absolute spectral irradiance (95 and 53 µW/cm^2/nm at 32- and 16-watts, respectively). Cold white light tubes emitted a full-spectrum of visible light (400–700 nm), consistently strong in the yellow region of the spectrum (peak 579 nm) with measured peaks of c. 35 µW/cm^2/nm (32-watts) and c. 20 µW/cm^2/nm (16-watts), but also with intense narrow peaks at 404, 435 and 546 nm and a colour temperature of approximately 5100 Kelvin.

Effect of light type

A total of 19,023 insects were trapped over the eight nights of the study. Control traps with non-operating lights caught minimal numbers of insects (nine aquatic insects, 137 terrestrial insects; < 0.8% of total catch) and are not considered further. In contrast, all operational light types attracted large numbers of aquatic and terrestrial adult insects belonging to the insect orders of interest, although catches varied considerably within treatments (Figure 3.3).

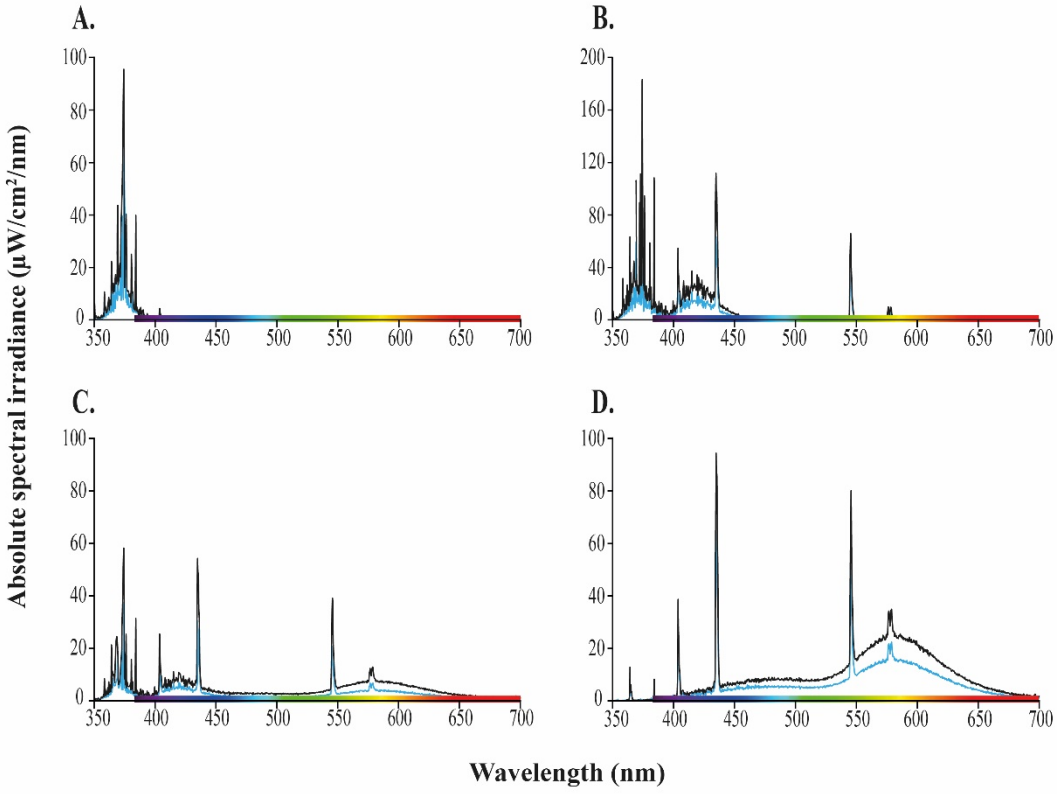

Figure 3.2 Spectral irradiance for the four fluorescent light types used in the study. A = blacklight-blue, B = blacklight, C = combination blacklight and cold white light, D = cold white light. The light blue line is the 16-watt spectral trace and the black line is the 32 watt spectral trace. The coloured bar along each x-axis represents relative position of visible light spectra.

The abundance of insects was significantly influenced by light type for most insect orders, and for both aquatic and terrestrial insect groups (Table 3.2). However, only a small to moderate proportion of the variation was attributable to the fixed effect of light type; random effects of sampling site and date contributed more strongly than light type (Table 3.2).

Cold white (CW) was the least effective light type, attracting significantly fewer individuals of all insect orders except Plecoptera, including when insects were placed in their respective groupings (aquatic insects and terrestrial insects). The lack of a light type effect for Plecoptera may have been a consequence of the small numbers caught, all of which were *Stenoperla prasina* (Newman, 1845).

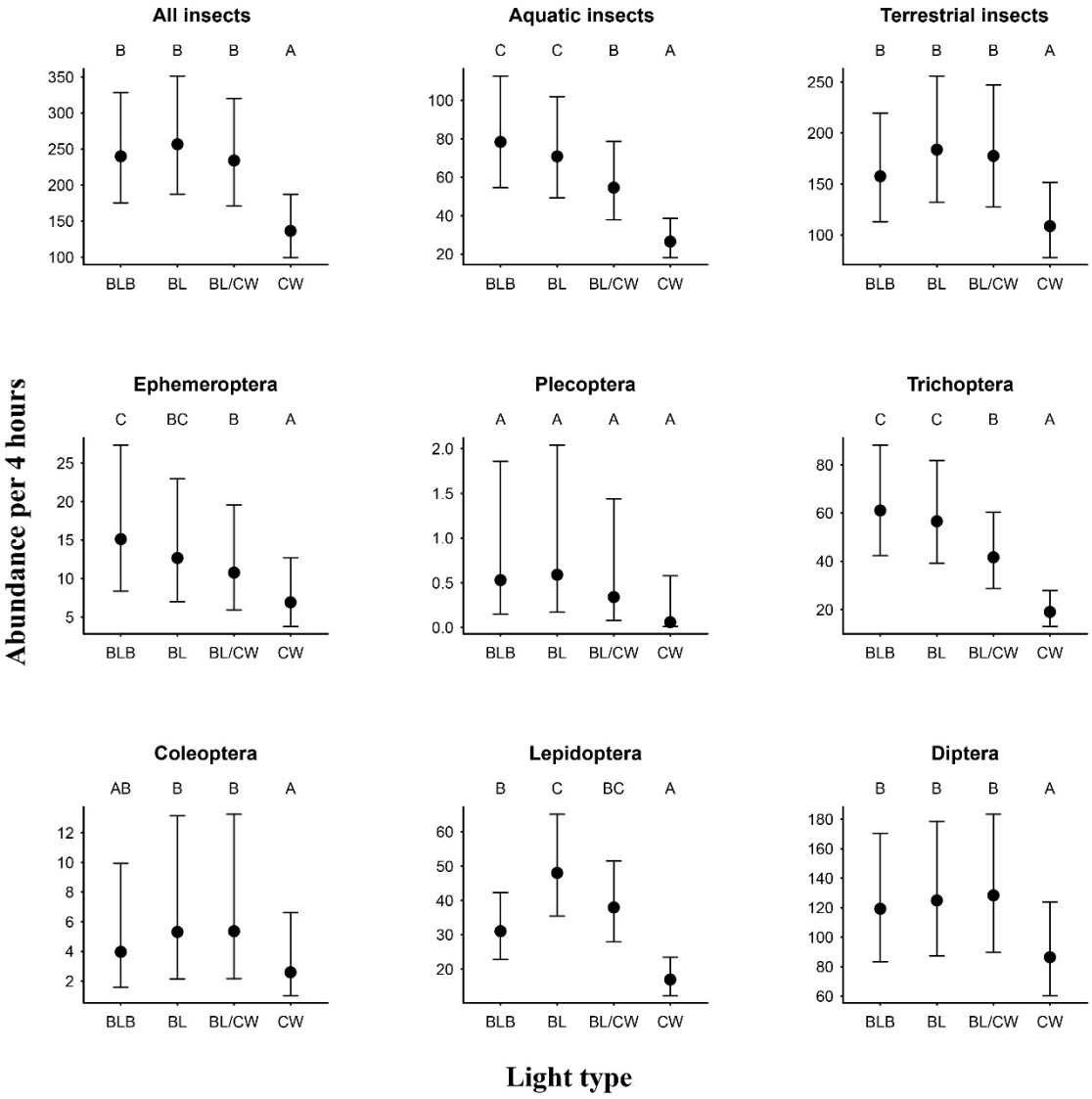

Figure 3.3 Predicted mean abundances of the modelled data (± 95% confidence intervals, N = ten for each) for insects caught over a four hour period with the four light types. BLB = blacklight-blue, BL = blacklight, BL/CW = combination blacklight and cold white light, CW = cold white light. Data obtained by traps fitted with two or four tubes have been combined (see Methods). Light types with different letters above the bars are significantly different ($P < 0.05$; Tukey's HSD tests).

Traps fitted with blacklight-blue (BLB) or blacklight (BL) tubes attracted about three times more aquatic insects, and about 1.5 times more terrestrial insects, than CW lights. Aquatic insect catches were generally greater in BLB light traps and terrestrial insect catches generally greater in BL light traps. However, the numbers of Ephemeroptera, Plecoptera, Trichoptera, Coleoptera and Diptera caught, and the numbers of combined aquatic and combined terrestrial groups, did not differ significantly between BL and BLB lights ($P > 0.05$). In contrast, significantly more Lepidoptera were taken by BL than BLB lights ($P < 0.01$).

Table 3.2 Results of generalised linear mixed-effects model tests of relationships between light type and two response variables, insect abundance and species richness (SR). N = number of samples. LRT = likelihood ratio test. Marginal R^2 and conditional R^2 values reflect the proportion of variation due to fixed effects alone and combined fixed + random effects, respectively.

Response variable	N	LRT	*P*	Marginal R^2	Conditional R^2
All insects	40	31.3	<0.001	0.28	0.84
Aquatic insects	40	50.1	<0.001	0.46	0.89
Terrestrial insects	40	18.7	<0.001	0.19	0.77
Ephemeroptera	40	18.6	<0.001	0.14	0.85
Plecoptera	40	6.7	0.08	0.26	0.59
Trichoptera	40	50.9	<0.001	0.51	0.89
Lepidoptera	40	28.4	<0.001	0.49	0.79
Coleoptera	40	8.2	0.04	0.13	0.83
Diptera	40	11.0	0.01	0.11	0.74
Ephemeroptera SR	40	3.0	0.40	0.09	0.29
Coloburiscus humeralis	40	1.3	0.74	0.15	0.65
Deleatidium angustum	40	1.3	0.73	0.03	0.52
Isothraulus abditus	40	28.2	<0.001	0.37	0.88
Zephlebia versicolor	40	6.0	0.11	0.07	0.66

Catches of most insect orders and groups did not differ significantly between traps fitted with BLB or BL lights and those fitted with the combination blacklight and cold white light (BL/CW). However, catches of Ephemeroptera, Trichoptera and aquatic insects grouped, were about 1.5 times greater in BLB traps than in BL/CW traps ($P < 0.05$) (Figure 3.3).

Effect of light intensity

Although 32-watt lights tended to attract more insects than 16-watt lights, differences in the size of catches were not significant ($P > 0.05$) for all insects combined, the three aquatic orders combined, the three terrestrial orders combined, or any single order-level group. Of the four ephemeropterans considered at the species level, a significant difference in catch size with light intensity was found for *Coloburiscus humeralis* only, with about twice as many individuals captured in 32- than 16-watt traps ($P = 0.043$).

Ephemeroptera species richness

Twenty six species of mayflies have been previously recorded from Pukenui Forest, from a range of habitats and temporal collections, using multiple methods (S. R. Pohe, unpubl. data; Supplementary Table S3.1). Fifteen species were collected during this study, 14 in blacklight-blue traps, 12 in blacklight traps, 12 in the combination BL/CW light traps, and nine in cold white light traps. Mayfly catches included both imaginal and subimaginal life stages, as well as males and females. No species were caught

in large numbers (Table 3.3), ranging from one individual of *Mauiulus luma* Towns & Peters, 1979 caught in a BLB light trap to 140 *Zephlebia versicolor* (Eaton, 1899) caught in traps of all four light treatments. Catch sizes of three of the four numerically dominant ephemeropteran species (*C. humeralis*, *Z. versicolor* and *Deleatidium angustum* Towns & Peters, 1996) showed no significant differences with light type, however, numbers of *Isothraulus abditus* Towns and Peters, 1979 were significantly lower in CW traps than all other light types ($P < 0.05$; Tukey's HSD test) (see Tables 3.2, 3.3).

Table 3.3 Total numbers of the four most numerous ephemeropteran species, all other Ephemeroptera and total Ephemeroptera collected during the study with four light types. BLB = blacklight-blue, BL = blacklight, BL/CW = combination blacklight and cold white light, CW = cold white light. Light traps were equipped with either two (16-watt) or four (32-watt) fluorescent tubes.

Species	BLB		BL		BL/CW		CW	
	16W	32W	16W	32W	16W	32W	16W	32W
Coloburiscus humeralis	10	11	6	20	13	14	3	16
Deleatidium angustum	11	10	10	2	8	16	6	11
Isothraulus abditus	46	26	15	17	11	13	1	0
Zephlebia versicolor	28	15	18	27	8	14	12	18
All other Ephemeroptera	14	24	16	20	11	21	15	8
Total Ephemeroptera	109	86	65	86	51	78	37	53

Discussion

Of the four lights compared in our study, blacklight-blue (BLB) and blacklight (BL) tubes caught approximately three times more aquatic insects than cold white light (CW), and about 1.5 times more terrestrial insects than CW. Catch numbers using the combination light (BL/CW) were generally intermediate in size and given that CW performed poorly, BL likely generated most of the BL/CW catch. Belton and Pucat (1967), studying *Culicoides* spp. (Diptera : Ceratopogonidae), also found that fluorescent blacklights (4-watt) were highly effective, with catches up to 40 times greater than those made with white and incandescent lights of comparable intensity. Similarly, Walker and Galbreath (1979) found that 8-watt BLB lights attracted twice as many insects (the same six orders as presented in this study, plus Hymenoptera) as white lights.

At 16- and 32-watts of light we obtained little evidence that use of the higher intensity attracted more insects, a finding consistent with that of Heath (1966) who concluded that there was little advantage in using 15-watt over 6-watt blacklights for collecting Lepidoptera. In our study, doubling the wattage only increased the spectral power by c. 55% (see Figure 3.2) and is perhaps one reason why differences in insect abundance obtained with two and four tube light traps were not significant. Nevertheless, a substantial increase in light intensity can be expected to result in an increase in catch size as found by Walker and Galbreath (1979) who obtained nearly twice as many insects using a 160-watt

'blended light', than with an 8-watt BLB lamp. Our needs required a portable set-up and thus high intensity lights requiring large power sources were not of interest.

Fifteen species of mayfly were recorded during the study; however, 26 species of mayflies have been previously recorded from Pukenui Forest. Our study was conducted early in the aquatic insect flight season out of necessity and we acknowledge some species were likely not yet on the wing.

Although our study indicated that blacklight-blue and blacklight were equally effective at attracting Ephemeroptera and Trichoptera (aquatic insects), we suggest that blacklight-blue lights be used in studies of adult aquatic insects, as they showed a reduced likelihood of attracting non-target terrestrial species. A reduction of bycatch of terrestrial insects is a favourable outcome with regard to faunal conservation and, more practically, sample sorting. Furthermore, our results suggest that for studies in remote locations, 16-watt lights should be sufficient to obtain reasonable numbers of specimens and, importantly, reduce the need to carry additional equipment, including heavy batteries.

Technical aspects and implications

With over 400 trap nights of light trapping and catch processing undertaken by one of us (SRP) it is clear that trap design and placement are critical for obtaining reasonable catches. Site specific environmental conditions (including river/stream conditions and habitat for aquatic insects), natural features and topography, vegetation density, and potential insect flight paths are important considerations. Further information on available techniques and equipment are well covered in a recent document by Patrick (2016) so are not discussed further here.

While there is limited technical data available for individual models of T5 fluorescent tubes (the type used in this study), it is known that performance of fluorescent tubes is dependent on tube temperature. In general, for standard T5 fluorescent tubes the optimal tube temperature (the temperature of the glass tube, not the ambient environment) is around 35 °C (but some tubes are 40–50 °C). Thus, when operating lights in cool environments the tubes may not attain optimal temperature, and their efficiency will decline. For example, if a tube works optimally at 35 °C but only achieves a glass temperature of 20 °C, maximum light output will be c. 70%, and at 10 °C less than 40% (technical data, The Light Edge Incorporation 2016). Environmental temperature will therefore have implications for the effectiveness of light trapping by entomologists working outdoors at night, particularly at higher latitudes or altitudes.

As well as tube temperature, age of the tube governs light efficiency, with older tubes performing more poorly. Thus, Philips (technical datasheet TL8W-05) reported a depreciation of 25% after 2000 hours for their 8-watt blacklight. Users also need to be aware that the peak wavelength of tubes varies among manufacturers, and for ultraviolet tubes is governed by the composition of the phosphor

compound within the tube, as well as by the type of filter coating manufacturers place on the tube. While these matters are unlikely to be of immediate concern to entomologists generating faunal lists, or collecting specimens for taxonomic purposes, they may have relevance for comparative ecological studies, particularly those that include temporal components.

Flight activity of many insect groups is directly related to air temperature and Ward et al. (1996) suggested the minimum air temperature threshold for flight of New Zealand Trichoptera was approximately 9 °C. During 2013–2015, in an extensive series of light trapping surveys for aquatic insects by one of us (SRP), Trichoptera and Lepidoptera were seldom caught when air temperature was < 10 °C, and Ephemeroptera at < 12 °C (S. R. Pohe, unpubl. data); a large and diverse range of insects were, however, generally obtained above 15 °C. Smith et al. (2002) also found that the largest catches of Trichoptera, taken in both forest and pasture landscapes, were obtained when air temperature was above 14 °C.

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Supplementary material

Table S3.1 Ephemeroptera recorded at the five stream sites during this study (1–5), and a complete list of species recorded previously from within the study area of Pukenui Forest, Northland (S. R. Pohe, unpubl. data). N = nymph/s by benthic sampling, I = imago or subimago by light trapping.

Species	(1) Pukenui Stream	(2) Kauri Stream	(3) Ark Stream	(4) Mangere Stream	(5) Pekapeka Stream	Pukenui Forest
Acanthophlebia cruentata (Hudson, 1904)	I	I		I	I	N, I
Ameletopsis perscitus (Eaton, 1899)	I	I	I	I	I	N, I
Arachnocolus phillipsi Towns & Peters, 1979	I	I				N, I
Austroclima sepia (Phillips, 1930)						N
Austronella planulata (Towns, 1983)						N
Coloburiscus humeralis (Walker, 1853)	I	I	I	I	I	N, I
Deleatidium (*D.*) *angustum* Towns & Peters, 1996	I	I	I	I	I	N, I
Deleatidium (*D.*) *cerinum* Phillips, 1930						I
Deleatidium (*D.*) *lillii* Eaton, 1899						N, I
Ichthybotus hudsoni (McLachlan, 1894)						N, I
Isothraulus abditus Towns & Peters, 1979		I	I	I	I	N, I
Mauiulus luma Towns & Peters, 1979				I		I
Neozephlebia scita (Walker, 1853)		I	I			N, I
Nesameletus flavitinctus (Tillyard, 1923)		I	I			N, I
Nesameletus ornatus (Eaton, 1883)				I		N, I
Oniscigaster wakefieldi McLachlan, 1873						N

Rallidens mcfarlanei Penniket, 1966						I
Siphlaenigma janae Penniket, 1962						N, I
Tepakia caligata Towns & Peters, 1996						N
Zephlebia borealis (Phillips, 1930)	I	I	I	I	I	N, I
Zephlebia dentata (Eaton, 1871)				I		N, I
Zephlebia inconspicua Towns, 1983						N, I
Zephlebia aff. pirongia sp. 1						N, I
Zephlebia spectabilis Towns, 1983	I				I	N, I
Zephlebia tuberculata Towns & Peters, 1996		I		I	I	N, I
Zephlebia versicolor (Eaton, 1899)	I	I	I	I	I	N, I

Chapter 4 – *Distribution of New Zealand Ephemeroptera*

"There are many hypotheses in science which are wrong.

That's perfectly all right: it's the aperture to finding out what's right.

Science is a self-correcting process."

Prof. Carl Sagan, 1980.

A failed attempt to negotiate difficult terrain during mayfly surveys on the Rakeahua River, Stewart Island. While conducting fieldwork I have fallen down stream banks, slipped on periphyton-covered substrate, and tripped head first into a deep stream pool while carrying 50 kg of equipment. If science is a "self-correcting process", perhaps I am a slow learner, or just not cut out for science!

Introduction

New Zealand has 57[‡] described species of Ephemeroptera, belonging to 20 genera in eight families (Hitchings & Hitchings 2018; Pohe 2018). They have close phylogenetic relationships with the cold-adapted mayflies of southern South America and Australia which, like New Zealand, broke away from the ancient Gondwanan continent (Edmunds 1972; Tsui & Peters 1975). Our current understanding of the distribution of the New Zealand mayfly fauna is primarily based on the comprehensive *Fauna of New Zealand* volumes by Towns and Peters (1996) and Hitchings and Staniczek (2003), records of specimens held in museum collections (including Hitchings 2001; Hitchings et al. 2015) and the summary publications by Hitchings (2008) and Macfarlane et al. (2010). Original taxonomic descriptions of some species also contain distribution information, particularly those published in the last four decades. A number of species-specific or location-specific studies have also contributed to our knowledge of mayfly distributions (Wisely 1952; McLean 1967, 1970; Chisholm 1984; Towns 1987; Hitchings 2005; Pohe 2008; Hitchings 2009) as well as national stream monitoring records prepared for the government's Ministry for the Environment (MFE 2015). However, to my knowledge no attempt has been made to undertake a nationwide survey at a specific point in time.

The recorded distribution of New Zealand mayfly species has been said to reflect both the country's past geological and climatic history, and the changing availability of present-day habitat (Hitchings 2008). Towns and Peters (1996) reported that more mayfly species were known from North Island streams than from similar streams in the South Island. Furthermore, within the North Island more species were known from lowland than high-altitude streams (Towns & Peters 1996). At the global scale a considerable amount of literature describes high species richness of insects in the tropics and a decline in richness at higher latitudes and altitudes (Rohde 1992, and references within; Horne et al. 2018). Therefore, it is of interest to investigate whether New Zealand mayfly species richness also fits the latitudinal and altitudinal gradients reported by others.

New Zealand is well suited for undertaking a latitudinal study as it is a narrow country whose three main islands extend over almost 13 degrees of latitude, and the latitudinal temperature cline (Figure 4.1) exhibits a significant decreasing temperature response to increasing latitude (i.e. from north to south; $R^2 = 0.77$; $F_{1, 27} = 90$; $P < 0.001$). The country also possesses substantial mountain ranges and reasonable access to about 1000 m a.s.l. enabling altitudinal analyses. Air temperature decreases with altitude (known as the temperature lapse rate) and in elevational gradients of mountain ecosystems is generally -0.6 to -0.8 degrees per 100 m increase in altitude (Rolland 2003). However, this rate varies significantly

[‡] The two most recent species were described by Hitchings and Hitchings (2018) however their very recent description has meant they are not included in analyses made in this chapter.

with respect to both local topography and meteorological conditions (Hodkinson 2005), and in New Zealand mean annual lapse rates of -0.5 degree per 100m have been reported (Norton 1985).

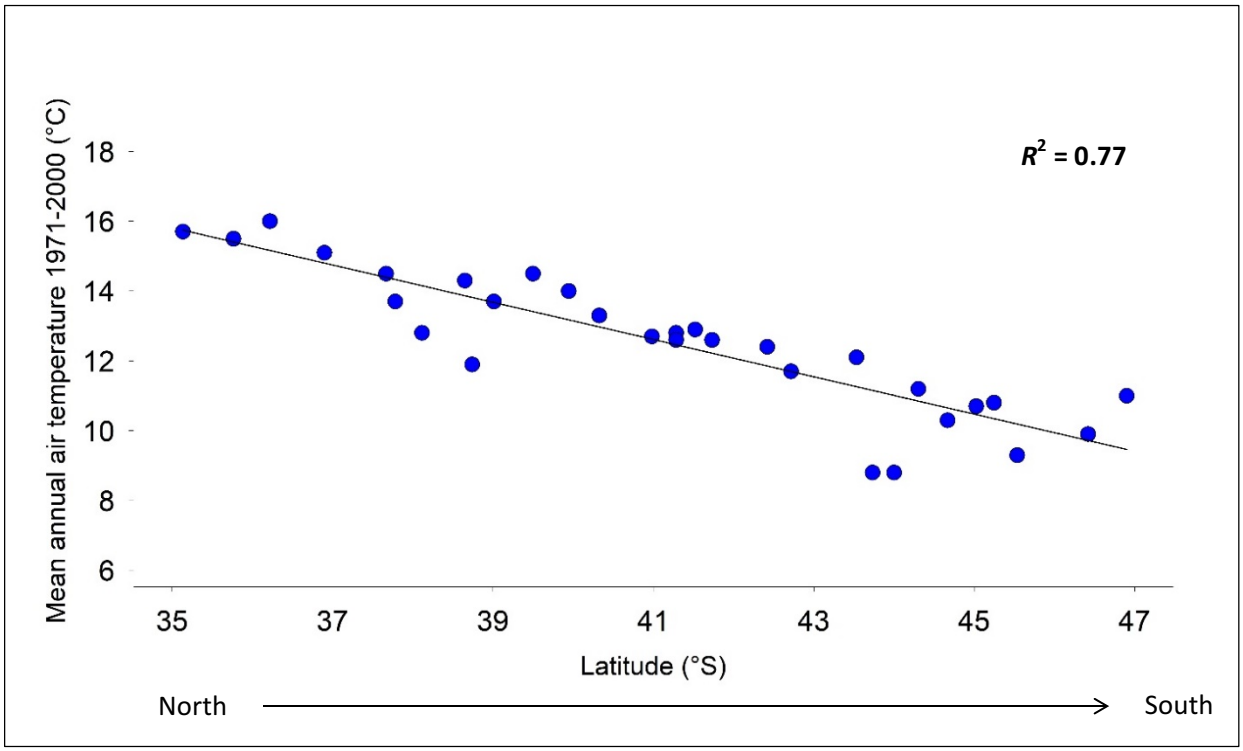


Figure 4.1 Mean annual temperature (1971–2000) across 29 New Zealand locations, arranged from north to south. Data source: http://cliflo.niwa.co.nz.

Temperature is a key physical parameter affecting the growth, metabolism, reproduction, emergence and distribution of aquatic insects (Hynes 1970). In ectotherms, body size is commonly correlated with temperature such that larger species occur in colder climates, while within species, size increases at higher latitudes and altitudes (e.g. Atkinson 1994). In their recent review of studies that examined temperature–body size (T–S) relationships in insects, Horne et al. (2018) found that a latitudinal T–S relationship was commonly seen in species from 12 orders, but altitudinal T–S relationships varied greatly between different taxa, and can be confounded by other environmental variables. No mayflies were included in the review by Horne et al. (2018) but the one caddisfly species and five of six stonefly species showed increases in size as altitude increased.

The aims of this chapter were to undertake a comprehensive assessment of the distribution of the New Zealand mayfly fauna in order to clarify the range and extent of each species, provide greater insight into the relative commonness and rarity of species and potentially document new species previously unknown to science. Specifically, I planned to:

1). Increase our knowledge of the present-day distribution of all described New Zealand mayfly species, based on a comprehensive survey throughout the length of the country, using standardised methods within a defined timeframe.

2). Assess whether new species might exist.

3). Compile a comprehensive record of New Zealand mayfly distributions based on a nationwide survey and reliable existing records from museums and other collections.

4). Test whether latitudinal and altitudinal distributions were significant drivers of mayfly diversity in New Zealand.

Methods

To assess distribution patterns of New Zealand mayflies, two methods of data collection were employed. Firstly, an investigation of present-day distributions was carried out with a comprehensive one-off nationwide survey. It consisted of a balanced spatial design and used established and standardised sampling methods. The faunal surveys targeted both nymphal and adult stages, and provided a robust and standardised dataset. The survey took three years to complete, implemented each austral summer from December–February.

In addition to the nationwide survey, material and records in three New Zealand museum collections, and the national State of the Environment macroinvertebrate database, were examined or assessed. The museum collections, all independently managed, and only one (Canterbury Museum) having an active curator of Ephemeroptera, have led to a fragmented record. The national collections were visited to verify the accuracy of identifications and collection records, and so draw as accurate and complete a picture of known New Zealand mayfly distributions. This multi-source dataset will form the basis of an inaugural New Zealand mayfly database.

Method 1 - The nationwide survey

Site selection

The three main Islands of New Zealand (North, South and Stewart Islands) extend across 13° of latitude. For clarity, three spatial scales are referred to throughout this chapter with regard to survey design and sample collection: latitudinal **'zones'**, regional **'locations'** and sampling **'sites'**. At the coarsest scale, the survey design was packaged into seven latitudinal **'zones'**, numbered from north to south, and each extending across 2° of latitude (Table 4.1, Figure 4.2). These latitudinal boundaries were extended around Cape Reinga, Coromandel Peninsula, and Southland coastline to encompass comparable adjoining land (see Figure 4.2). Specifically, the northern boundary near Cape Reinga was extended so that all of northern New Zealand was included in the sampling location selection process. Similarly, the northern zone boundary was extended to include all of the Coromandel Peninsula, rather than have a small part of it in Zone 1. Finally, the southern boundary of Zone 6 at the bottom of the South Island was extended to incorporate the whole of Southland.

The landmass within Zone 7 only comprised only about 1° of latitude (in contrast to 2° in the other zones) due to it being an island (Stewart Island), and also the southern extent of the country. To account for this during selection of sampling locations, the number of tentative sampling locations was reduced, in proportion to land area within the zone.

Table 4.1 The seven latitudinal zones and their ranges. The main geographic regions included in each zone are shown for general context.

Latitudinal zone	Regions	Latitudinal range
Zone 1	Northland	34° 30′ S – 36° 30′ S (plus 6′ extension)
Zone 2	Auckland/Waikato/Bay of Plenty	36° 30′ S – 38° 30′ S (plus 3′ extension)
Zone 3	Taranaki/Manawatu/Hawke's Bay	38° 30′ S – 40° 30′ S
Zone 4	Wellington/Tasman/Marlborough	40° 30′ S – 42° 30′ S
Zone 5	West Coast/Canterbury	42° 30′ S – 44° 30′ S
Zone 6	Otago/Fiordland /Southland	44° 30′ S – 46° 30′ S (plus 12′ extension)
Zone 7	Stewart Island	46° 30′ S – 48° 30′ S

The primary purpose for including zones in the sampling location selection process was to ensure that comparable numbers of sampling locations (and therefore biological representativeness) would be sampled across the entire country, and that they would enable realistic comparisons to be made in relation to latitude and altitude. The following criteria were used to select specific sampling 'locations' within each zone:

1). Locations needed to be positioned in 'natural' areas. Thus, only streams occurring in predominantly unmodified landscapes were considered, i.e. forest or mountainous habitats in order to avoid confounding factors associated with urbanisation, agricultural inputs, and habitat modification. Suitable locations were identified using geographical information system (GIS) topological and aerial maps and overlaid with land-use (e.g. native forest, exotic forest, pasture) and cadastral (e.g. conservation administered land) shapefiles.

2). Locations needed to be as representative as possible of their general locations and encompass as much of the range of natural stream variability as possible within each zone. Initially, potentially suitable locations within each latitudinal zone were identified based on the 20-group level GIS data layers of the Freshwater Ecosystems of New Zealand (FENZ) geodatabase (Leathwick et al. 2010). The FENZ is a classification system that describes environmental conditions across all of New Zealand's rivers and streams and is based on the spatial data topology (flow positions and segments) developed for the River Environment Classification (REC) of Snelder and Biggs (2002). The FENZ steam classification was produced by combining 23 stream environmental variables (e.g. aspects of physical nature, climate, up-stream catchment vegetation) and biological data (native freshwater fish and macroinvertebrate records). Stream locations that were selected for my survey

represented the full range of environmental stream-types within each latitudinal zone, in proportion of their occurrence within that zone (Table 4.2).

3). Locations needed to be spatially distributed within each zone. This required east–west and north–south considerations, and a range of altitudes to be included within each zone. In general, ranges of altitudes were already accounted for via the use of the FENZ system in Criterion 2.

4). Locations needed to be accessible. For logistic reasons realistic access to sampling locations was needed. Thus, streams (stream segments) matching criteria 1–3 were required to be within 5 km of a road or vehicle track, or on or near a public walking track, and preferably not on private land as it was too difficult to obtain permission to work on it in many parts of the country.

Streams that met all four criteria listed above were considered for use in the nationwide survey, and were generally on conservation, or Queen Elizabeth II National Trust administrated land. Within each zone a provisional short-list of 20 locations was selected and given names based on near-by landmarks (e.g. a town, mountain range or pass, forest, lake, conservation land title). I expected that some of these locations would be unsuitable for sampling when the location was visited, but that about 15 **'locations'** per zone would be suitable for the study.

At all locations, three sampling **'sites'** were sampled, an attempt being made to target a range of habitat and flow regimes by positioning them on second, third and fourth order streams (i.e. one on each), within a single catchment. If this was not possible, sites were selected on main-stem streams (generally stream order 3), and/or along first or second order tributaries when present. In all cases, sites were well separated; the distance between them being dictated by the nature of the stream systems, and the surrounding topography and vegetation. The median distance between sites was 140 m; minimum 30 m; maximum 2,290 m. A total of 81 locations comprising 243 sites were sampled (Figure 4.3; Appendix 1). Zone 7 (Stewart Island), had only three sampling locations, due to its proportionately small land area, and due to the costs and logistics involved in sampling on the island.

Permits and permissions

Permissions were obtained for access and sample collection at all short-listed sampling locations. These included research collecting permits from the Auckland Council (permit CS56; 2 locations) and Department of Conservation (permits 37682-RES, 45516-RES, CA-28545-FAU, WC-26300-FAU; 99 locations), and approvals from Queen Elizabeth II National Trust representatives (7 locations) and private or Trustee landowners (18 locations). Consultation was also undertaken with local iwi (indigenous Maori community members) for many of the sampling locations, particularly in the North Island.

Survey procedure

The field survey was carried out during the expected peak emergence period for winged New Zealand mayflies (austral summer) and was completed in three stages: North Island, 22/11/13–21/02/14, South Island (except Otago/Fiordland), 22/11/14–27/02/15 and Otago/Fiordland and Stewart Island, 02/12/15–28/01/16). Surveys at each location were carried out over two days. At each site a light trap was set and physico-chemical measurements of the stream environment made.

Because the surveys included light trapping for adult stages, suitable weather conditions were critical, as imaginal and subimaginal mayflies are less likely to be on-the-wing when conditions are wet and windy, or cold (< 13°) . Therefore, when conditions were deemed unsuitable, sampling was halted until conditions were favourable, and unsuccessful sampling events were either repeated on another occasion, or omitted from the results**.** A successful sampling event was easily determined, with catches obtained always presenting either excellent catches (100–300 individuals) or very poor catches (0–20 individuals).

Ultraviolet fluorescent lights comprising four [§] BLB 8-watt tubes (32 W; 350 nm) were used for sampling (see Chapter 3). The lights were positioned above 9L, white trays (50x36x5 cm), half-filled with water to entrap arriving mayflies (Figure 4.4). Biodegradable Surfax® detergent (30 ml) was added to the water to reduce surface tension, thereby enhancing the retention of trapped insects. Lights were powered by two 12-volt batteries (7–9 a/h) run in parallel, and activated by 24-hour timing modules set to activate at sunset and deactivate four hours later. This period of operation was in accordance with the findings of Norrie (1969) and Ward et al. (1996) who suggested that flight activity of New Zealand mayflies and caddisflies was highest at that time. Traps were placed on the ground alongside streams, or on a large boulder mid-stream, but always within 3-metres of the water. Traps were raised at least 1-metre above the stream to improve visibility and access for arriving insects, but also in case of rising stream levels due to overnight rain.

[§] Due to equipment damage, several surveys were undertaken using three BLB and one BL tube.

Table 4.2 Number and type of stream segments / proportions occurring per zone, and number of samples in each FENZ class per zone (in parentheses and blue text), for the nationwide survey. Values are based on the FENZ 20-group level environmental stream-types of Leathwick et al. (2010) and a sampling design of 15 samples per zone for zones 1–6, and 3 samples for Zone 7 (due to reduced land area and increased logistics). To calculate number of samples in each FENZ class per zone, only numerical components ≥ 0.5 were considered, and were rounded up to whole numbers.

FENZ	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	Zone 7
A	17,933 / 60.1 (9)	21,598 / 27.6 (4)	24,143 / 21.3 (3)	9,533 / 9.7 (2)	15,812 / 14.4 (2)	20,502 / 15.4 (2)	
B	79 / 0.3 (< 0.1)	950 / 1.2 (0.2)	35 / 0.03 (< 0.1)	28 / 0.03 (< 0.1)	232 / 0.2 (< 0.1)	465 / 0.3 (< 0.1)	20 / 0.7 (< 0.1)
C	11,847 / 39.7 (6)	55,629 / 71.0 (11)	81,152 / 71.5 (11)	61,142 / 62.2 (9)	31,789 / 29.0 (5)	8,902 / 6.7 (1)	
D				37 / 0.04 (< 0.1)	6,885 / 6.3 (1)	15,558 / 11.7 (2)	
E					272 / 0.2 (< 0.1)	668 / 0.5 (< 0.1)	
F		48 / 0.06 (< 0.1)	14 / 0.01 (< 0.1)	24 / 0.02 (< 0.1)	462 / 0.4 (< 0.1)	53 / 0.04 (< 0.1)	
G		40 / 0.05 (< 0.1)	5,300 / 4.7 (1)	2,809 / 2.9 (0.4)	5,474 / 5.0 (1)	41,084 / 30.8 (5)	2,581 / 88.1 (3)
H		98 / 0.1 (< 0.1)	2,365 / 2.1 (0.3)	12,073 / 12.3 (2)	14,155 / 12.9 (2)	16,891 / 12.7 (2)	69 / 2.4 (< 0.1)
I				360 / 0.4 (< 0.1)	2,541 / 2.3 (0.3)	184 / 0.1 (< 0.1)	
J		9 / 0.01 (< 0.1)	60 / 0.05 (< 0.1)	6,612 / 6.7 (1)	9,893 / 9.0 (2)	11,187 / 8.4 (1)	4 / 0.1 (< 0.1)
K					572 / 0.5 (< 0.1)	27 / 0.02 (< 0.1)	
L					1,150 / 1.1 (< 0.1)	144 / 0.1 (< 0.1)	
M					141 / 0. 1 (< 0.1)		
N			323 / 0.3 (< 0.1)	4,706 / 4.8 (1)	7,872 / 7.2 (1)	1,0265 / 7.7 (1)	189 / 6.5 (0.2)
O			19 / 0.02 (< 0.1)	175 / 0.2 (< 0.1)	3,838 / 3.5 (1)	3,393 / 2.5 (1)	61 / 2.1 (< 0.1)
P			25 / 0.02 (< 0.1)	532 / 0.5 (< 0.1)	2,599 / 2.4 (< 0.1)	2,788 / 2.1 (0.3)	3 / 0.1 (< 0.1)
Q			52 / 0.05 (< 0.1)	190 / 0.2 (< 0.1)	1,563 / 1.4 (< 0.1)	669 / 0.5 (< 0.1)	2 / 0.07 (< 0.1)
R					13 / 0.01 (< 0.1)	51 / 0.04 (< 0.1)	
S			4 / < 0.01 (< 0.1)	1 / < 0.01 (< 0.1)	2,764 / 2.5 (< 0.1)	351 / 0.3 (< 0.1)	
T					1,490 / 1.4 (< 0.1)	57 / 0.04 (< 0.1)	
Total	**29,859 / 100 (15)**	**78,372 / 100 (15)**	**113,492 / 100 (15)**	**98,222 / 100 (15)**	**109,517 / 100 (15)**	**133,239 / 100 (15)**	**2,929 / 100 (3)**

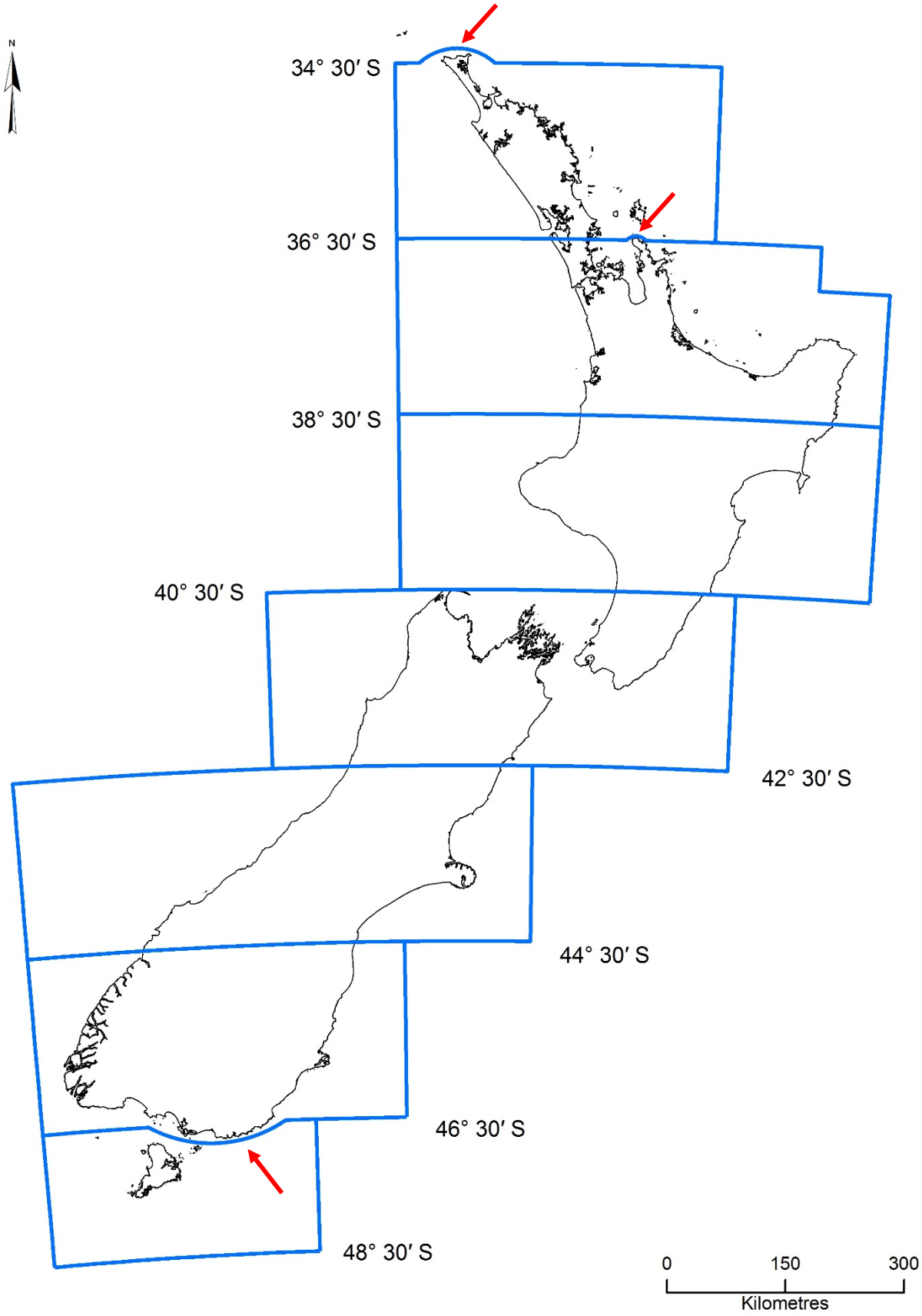


Figure 4.2 Positions of the 2° latitudinal lines chosen to delineate the zones used in the sampling location selection. Red arrows indicate where minor extensions were made to the latitudinal boundaries.

Figure 4.3 Positions of the 81 locations sampled successfully during the nationwide survey.

In addition to light trapping for imagos, nymphal stages were collected from a range of in-stream habitats. This was done by hand-picking specimens from rocks and wood, and using a hand-held net (500 µm mesh) swept through pools, trailing vegetation and leaf packs, and by kick-sampling in riffles and course-gravel margins of pools. One hour of benthic sampling was allocated per location, i.e. 20-minutes per individual site. Hand-netted samples were emptied into trays and mayfly specimens were picked in the field. Since the aim of sampling was to collect as many species as possible, and targeted sampling of differing habitats was important, samples cannot be considered to be quantitative. *In-situ* processing and preserving of nymphs was critical as they are extremely fragile, and fully intact specimens were required for accurate species-level identifications. All specimens (nymphs, subimagos and imagos) were preserved in 95% ethanol in the field, and stored individually in Axygen® microtubes. Individual storage ensured that body parts, particularly legs, were retained, and also to prevent potential cross-contamination between specimens that might be used for molecular analyses.

Figure 4.4 Light trap set-up consisting of four 350 nm blacklight-blue fluorescent tubes set over a pan trap. Lights were activated by a 24-hour timer module and powered by two 12-volt batteries.

Physical and chemical measurements

At all sites *in-situ* physico-chemical water readings were recorded using a YSI Professional Plus hand-held meter with Quatro multi-parameter sensor that recorded pH, dissolved oxygen (g/m^3 and % saturation), conductivity (µS/cm), salinity (ppt) and water temperature (°C). The meter was calibrated for oxygen readings daily at the sampling location and every two weeks for pH. Mean water velocity was estimated to be in one of six broad categories: 0 = <0.1 m/s, 1 = 0.1–0.2 m/s, 2 = 0.2–0.4 m/s, 3 = 0.4–0.6 m/s, 4 = 0.6–0.8 m/s, 5 = > 0.8 m/s.

At each location physical measurements of the stream environment were made within a 100m reach (50 m upstream and 50 m downstream of the light trap). Air and water temperature were logged overnight, for a 12 hour period (15 minute intervals), using Hobo® pendant data loggers (Model UA-002-64, Onset Computer Corporation). Wetted width of the stream channel was measured at five points using a laser rangefinder (Bosch PLR-50). Depths of up to five pools (number depending on presence) and ten riffle/runs were measured. Relative streambed substrate composition was assessed following the method of Wolman (1954), and modified by (a) recording particle size of 50 particles, size being measured as the greatest axis in mm, and (b) by selecting particles while pacing up the stream reach in a zig-zag manner. Hydrologic stability was assessed using the Pfankuch (1975) stability index, which incorporates 15 stability attributes of upper and lower banks, and the stream bed. Percent coverage of overhead canopy was measured with a convex spherical densiometer (Model-A, Forestry Suppliers incorporated; Lemmon 1956, 1957). Following the manufacturer's instructions, four readings (one at each of the primary compass points) were taken at each of three positions along the sampling reach to give a total of 12 readings. The mean of the 12 readings was multiplied by 1.04, to give a measure of overhead canopy cover i.e. one per location.

Specimen identification

Specimens were examined in the laboratory using a Nikon® 10–63x stereomicroscope (Model SMZ800) fitted with a 1x plan objective, and identified using the dichotomous keys of Phillips (1930, 1931), Towns and Peters (1996), Hitchings and Staniczek (2003) and Winterbourn et al. (2006), together with original taxonomic descriptions. Once identified, specimens were placed back into labelled microtubes with fresh 80–90% ethanol.

A number of difficult specimens, mainly females, subimagos and nymphs were examined by molecular methods (COI barcodes; see Appendix 2) to confirm species-level identifications that were deemed likely to influence species counts i.e. when several species of a genus were suspected to be present at the same location, but could not be positively identified by microscopy. Molecular methods were also used to confirm the identities of specimens of particular interest due to unexpected distributional ranges, general rarity, being of conservation interest, or were suspected to contain cryptic

species. They included *Rallidens* spp., *Zephlebia nebulosa*/*Z.* aff. *nebulosa* sp. 1, *Z. pirongia*/*Z.* aff. *pirongia* sp. 1, *Z*. sp. 1, *Deleatidium* (*D*.) *magnum* and *Siphlaenigma janae*.

To facilitate DNA-based identifications, genetic sequences of New Zealand mayfly species were required for comparison, however, very few publicly available sequences exist for New Zealand mayflies (but see GenBank at https://www.ncbi.nlm.nih.gov/genbank (Benson et al. 2013)). A number of private sequences were also made available from the Barcode of Life Data System (BOLD) (Ratnasingham & Hebert 2007) but were of variable quality, particularly with respect to taxonomic identification. Thus, a reference 'DNA library' of New Zealand mayfly sequences was developed to enable direct comparisons of genetic sequence results.

Genetic identifications and the 'mayfly DNA library'

To construct a reference DNA library of New Zealand mayflies, sequences of ~70% of the described New Zealand fauna were generated from positively identified specimens that were collected during my nationwide survey. Molecular methods and genetic analysis procedures used to develop the mayfly DNA library, and also to compare and contrast inter- and intra-specific pairwise genetic distances, are described in detail in Chapters 5 and 6 (Pohe et al. 2018; Pohe et al. 2019). In brief, mitochondrial DNA was extracted from legs of individuals and a fragment of the cytochrome *c* oxidase subunit I (COI) gene was amplified by polymerase chain reaction (PCR) using universal primers (Folmer et al. 1994). To facilitate the future construction of a New Zealand mayfly phylogeny, and for population structure analyses in Chapter 5, the D2 fragment of the 28S nuclear gene was also amplified. All PCR product was purified and sent to MacroGen (Seoul, South Korea) for sequencing. Sequences of both COI and 28S genes were aligned manually and trimmed to 658 and 833 base pairs (bp), respectively, using BioEdit Sequence Alignment Editor version 7.2.5 (Hall 1999). A total of 389 COI and 86 28S sequences were generated for the library, comprising 40 species and, when possible, representatives of each species from a number of locations across their distributional range (to help assess regional genetic variation). To enable comparisons to be made between DNA sequences, pairwise genetic distances were generated in Mega 7.0 (Kumar et al. 2016) using the uncorrected *p*-distance model (see Collins et al. 2012) which provides proportional differences of DNA nucleotides of two genetic sequences (obtained by dividing the number of nucleotide differences by the total number of nucleotides compared).

Method 2 - Historical data

Data acquisition

Mayfly distribution records obtained during the nationwide survey were combined with historical specimen records to improve the overall understanding of New Zealand's mayfly distributions. Historical data sources included specimens I collected prior to the nationwide survey (Pohe personal collection), 1995–2011 records from the New Zealand Ministry for the Environment's (MFE) freshwater invertebrate monitoring database (MFE 2015), specimens held in the Otago Museum, Canterbury Museum and New Zealand Museum (Te Papa), and type specimen records held in the New Zealand Arthropod Collection (Table 4.3). Only mayfly records that were identified to species level and had meaningful locality labels (locality label data that had, or could be converted to, latitude/longitude positioning coordinates) were considered for inclusion in the distribution dataset. Records in the MFE database generally provided data at the generic level; however, about 7,000 records (~30%) from that source were usable as they comprised data at the species level. Further, many New Zealand ephemeropteran genera are monospecific, and thus were able to be included. The MFE database is generated from information obtained by ecologists working for local-body councils, and is generally of good standard, with quality control procedures in place, though some degree of misidentification is possible. Generation of such a comprehensive New Zealand mayfly dataset, has not been produced before to the best of my knowledge, and I intend it to become the basis of an official New Zealand mayfly database.

Table 4.3 Data sources and specimen records used to determine New Zealand mayfly distributions.

Data Source	Number of specimen records
Pohe nationwide survey (this research)	18,021
Pohe personal collection (prior to this research)	3,183
MFE freshwater invertebrate monitoring data (1995–2011 only)	6,961
New Zealand Arthropod Collection type specimens	13
New Zealand Museum (Te Papa) Collection	239
Canterbury Museum Collection	45,006
Otago Museum Collection	36
Total	**73,459**

Museum collections listed above were visited during 2015–2016. Specimen records were verified, corrected if required, and entered into my database. All locality coordinates were also verified, by cross-referencing them with locality labels. Species distributions based on the collated data (including the nationwide survey data) were mapped using ESRI® ArcMap™ software (ESRI 2010). Due to a general lack of positioning coordinates, published literature was not included in the dataset, except where specimens in collections were also part of a published study, e.g. descriptions of type specimens.

Analysis of distribution data

Distributions of individual species are presented in relation to the system of aquatic ecoregions for New Zealand (Figure 4.5) proposed by Harding and Winterbourn (1997) and also the geographic regions (see Figure 2.1, Chapter 2) proposed by Crosby et al. (1998). The former were produced by merging climatic and geomorphological variables considered likely to influence stream biota, based on the hierarchical model of Biggs et al. (1990). The geographical regions proposed by Crosby et al. (1998) have a more arbitrary basis and were designed primarily to facilitate the categorising of terrestrial specimen localities. Their boundaries generally equate to broad climatic regions used in weather forecasting, and consequently incorporate climatic elements that influence biological distributions. I had no sampling locations within the BP, EH and MN ecoregions of Harding and Winterbourn (1997), or in the HB and WI biogeographical zones of Crosby et al. (1998), partly due to chance, and partly due to a lack of suitable habitat required to meet location selection criterion 1 (areas of naturalness). Biogeographic patterns displayed by mayfly species within New Zealand are described in the manner used by McLellan (2006) for New Zealand Plecoptera (stoneflies) and are based on combined survey and historical data. Species acknowledged as widespread are listed in the results (see 'Distribution patterns of individual species' section) with ^{W} and those as sparse with ^{Sp}. Species can also be widely distributed, but sparse over all or part their ranges ($^{W, Sp}$) or conversely, restricted to a particular area within that distribution (^{R}).

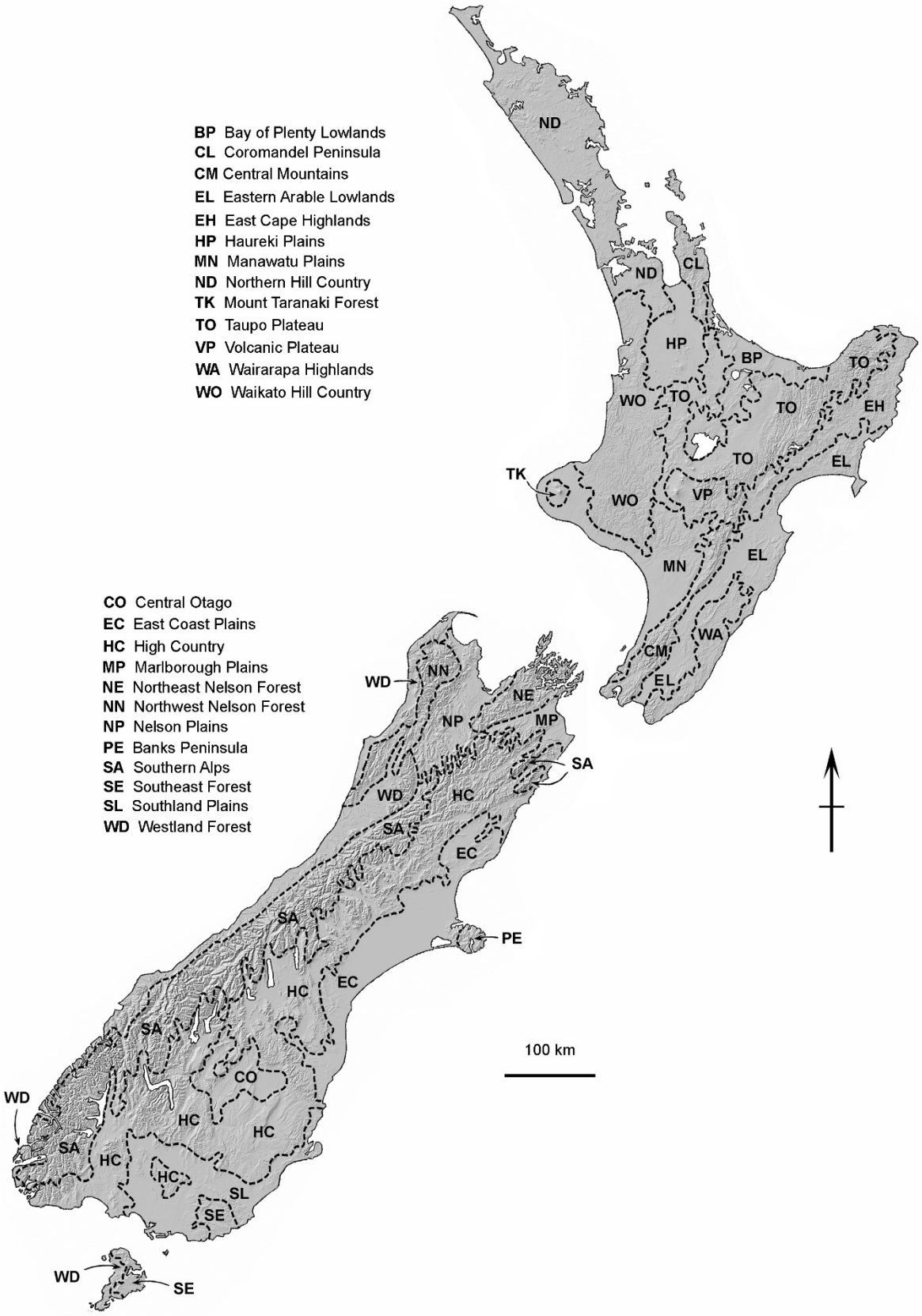


Figure 4.5 New Zealand aquatic ecoregions proposed by Harding and Winterbourn (1997). Reproduced from Craig et al. (2012) with the authors' permission. Copyright: Landcare Research New Zealand Ltd.

Analyses of latitudinal and altitudinal patterns of distribution

Three components of species diversity are commonly recognised in ecology: local alpha (α) diversity, differentiation or beta (β) diversity and total regional or gamma (γ) diversity (Koleff et al. 2003). In this research I refer to species richness of faunal samples from individual '**locations**' as α diversity, the change in species composition between clusters of locations, arranged into latitudinal '**zones**' or altitudinal '**bands**', as β diversity, and the total regional species richness within New Zealand, as γ diversity.

Analyses of relationships of species and faunal similarity with latitude and altitude were based on data obtained in the nationwide survey. Data from each site at a locality were pooled so that the data matrix used comprised the total number of species at each of 81 localities. For some analyses all locations within a latitudinal zone were pooled so that inter-zonal comparisons could be made. In comparing species richness with latitude and altitude, all species obtained by light trapping and in benthic collections were included. However, Stewart Island (Zone 7) was excluded from comparisons of faunal similarity among zones as sampling had only been done at three localities.

Margalef's Index (*d*), calculated as $d = (S–1)/\ln N$, where *S* is the total number of taxa and *N* is the total number of individuals (Margalef 1958; Boothroyd & Stark 2000), was also calculated as a second measure of species richness that took into account the total number of mayflies recorded in each collection. Because numbers of species collected can be expected to increase as the size of collections increases due to the greater likelihood of finding rare species, Margalef's Index is effectively a method of rarefaction (Ball et al. 2015). Because benthic mayfly collections were not sampled quantitatively, Margalef's Index was calculated from light trap data only.

Similarity of the mayfly fauna among latitudinal and altitudinal zones was assessed with the Sørensen (=Czekanowski or Bray-Curtis coefficient of some authors) distance measure, calculated manually as 2c/a + b, where c is species in common between two zones, and a and b are the total numbers of species in each zone (Sørensen 1948; McCune & Mefford 2011). Calculations were made with presence–absence data obtained by light trapping and benthic collecting. Whittaker's species turnover (β_w) provided a measure of beta diversity, i.e. the relationship between regional and local species diversity (richness). It is calculated as γ/α–1, where γ (gamma) = total species richness in the region of interest and α (alpha) = species diversity at a particular location (Whittaker 1960, 1972).

Nonmetric multidimensional scaling (NMS) incorporating the Bray–Curtis distance measure was used to ordinate invertebrate communities in taxonomic space for stream locations across New Zealand (81 locations, 47 species). Presence–absence data were used and the solution with the lowest stress in two dimensions was selected as only small reductions in stress were obtained with additional axes. Monte Carlo simulations (250 runs) were used to test the statistical significance of the ordination. NMS

in conjunction with the Bray–Curtis distance measure provides a robust method for analysing community data sets, which often have a high proportion of zeros, leading to non-normally distributed data (McCune & Grace 2002). NMS was run in the statistical software PC-ORD version 6.22 (McCune & Mefford 2011). In addition, the similarity percentage method (SIMPER) was used to identify which invertebrate taxa were most influential in determining regional differences (Monte Carlo test with 1000 runs), and was calculated in the statistical software package PAST version 3.2 (Hammer et al. 2001).

I also investigated whether size of the mayfly *Coloburiscus humeralis* was related to latitude and altitude. This species was selected for the purpose as it was the most commonly obtained species in light trap collections throughout the country, and was often numerically abundant. It was also a relatively large species which allowed meaningful changes in size to be detected. To obtain length measurements, a single wing was dissected from each specimen and placed in a syracuse dish with 90% ethanol. The wing was covered with a cover slip (to prevent movement and maintain a horizontal position) and photographed through a Leica stereomicroscope (Model: M125) with microscope camera (Model: Leica DFC295). Images were annotated with Leica Application Suite software and measurements captured with ImageJ version 1.49d image processing software (Rasband 1997–2014). Measurements were accurate to a few pixels (± 0.04 mm) using magnification functions of the software. Forewing length, measured from the apex of the wing to the point of attachment at the basal plate (Figure 4.6), was determined for 1318 imagos and subimagos (combined dataset) from the nationwide survey. Forewing lengths of imagos and subimagos did not did not differ in a subset of 271 individuals (72 imagos, 199 subimagos) from four geographically separated locations (Table 4.4).

Table 4.4 Summary table of forewing length comparisons between imago and subimago *Coloburiscus humeralis*.

Location (Crosby code)	Sex	Imago (*n* =)	Subimago (*n* =)	*t*-value	*P*-value
Waipoua (ND)	Male	5	69	0.03	0.98
	Female	33	33	1.5	0.13
Cascades (AK)	Male	0	0	–	–
	Female	10	33	0.9	0.40
Makohine (RI)	Male	3	14	0.5	0.61
	Female	10	17	0.9	0.34
Okuti (MC)	Male	6	23	1.0	0.31
	Female	5	10	0.4	0.68

Figure 4.6 The linear measurement used to determine forewing length of *Coloburiscus humeralis*.

Relationships between mayfly species richness and body size (as forewing length) with altitude were also carried out, as described above, for a separate study at a smaller geographical scale on Mount Taranaki in the North Island. Mount Taranaki was chosen because although altitudinal sampling could not be undertaken on a single stream, numerous similar sized stony streams flow down its cone-like form and roads provide access to them at various elevations up to about 1000 m. The mountain and its slopes of native vegetation also have conservation protection (Egmont National Park) thus providing a natural system with minimal human-induced effects on water quality. I undertook two light trapping surveys at seven 'locations' on the mountain (Table 4.5; Figure 4.7) in early summer (13–19 December 2015) and late summer (3–6 February 2016) using identical collecting methods to those employed in the nationwide survey. The only difference was that only one site was sampled per location (rather than three), for logistical reasons. All mayflies captured were identified and counted and used to compare species richness and community similarity among locations and times. Forewing length was determined for 166 *Coloburiscus humeralis* and used to compare mayfly size among altitudinal locations. All physico-chemical variables on Mount Taranaki were measured as in the nationwide survey (see 'Physical and chemical measurements' section).

Table 4.5 Sampling locations and their altitudes on Mount Taranaki. Names of the streams on which locations were positioned and stream widths at sampling sites are also given.

Location	Waterway	Stream width (m)	Latitude; longitude (NZGD 2000)
120 m	Waimoku Stream tributary	2.4	39° 08.9031′ S; 173° 56.2385′ E
280 m	Cold Stream	4.2	39° 23.8783′ S; 173° 59.9414′ E
420 m	Timaru Stream	5.5	39° 12.5344′ S; 173° 58.6967′ E
600 m	Waiongana Stream	5.6	39° 14.7196′ S; 174° 06.9387′ E
730 m	Camp Stream	3.4	39° 15.4149′ S; 174° 05.8406′ E
880 m	Kapuni Stream	3.8	39° 19.3575′ S; 174° 06.2869′ E
1100 m	Waingongoro River tributary	0.3	39° 18.6132′ S; 174° 05.7368′ E

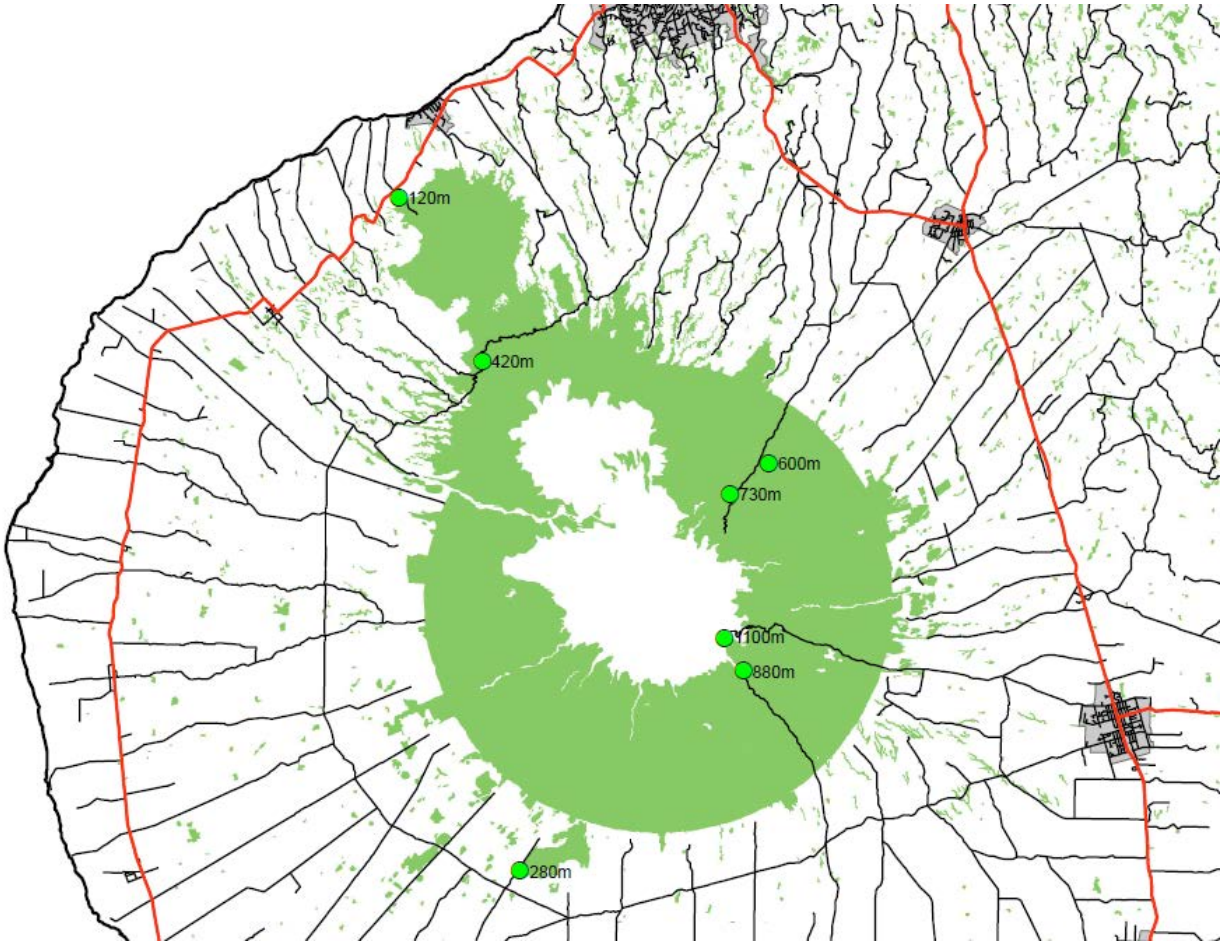


Figure 4.7 Positions of altitudinal sampling locations on Mount Taranaki. Dark green shading indicates native vegetation which clearly defines Egmont National Park. Red lines indicate major highways and black lines represent arterial roads. Gray zones represent townships.

Statistical tests and graphical relationships relating to species richness, faunal similarity and mayfly size with latitude and altitude were drawn and/or calculated with the statistical software packages 'R' (R Core Team 2018) or PAST3 (Hammer et al. 2001).

Results

The nationwide survey

Untangling the taxonomic identity of New Zealand's undescribed or poorly defined species was beyond the scope of this research. Therefore, identifications are primarily based on existing taxonomic descriptions and identification keys, and I acknowledge that some presently undescribed species may key out as described species. A total of 18,021 individuals comprising 47 of the 57 described species, and five tentative new species (total gamma diversity of 52 species) were collected during the nationwide survey. A summary table of physio-chemical data and the mayfly fauna obtained is presented in Table 4.6. As expected, air and water temperatures showed a gradual decline with increasing latitude and dissolved oxygen concentration was high in all zones. Conductivity tended to be higher in North Island streams, whereas pH was close to neutral or slightly alkaline in all zones except on Stewart Island where streams

Table 4.6 Summary of the latitudinal zone distributions of mayfly taxa and physico-chemical data. Distributions of leptophlebiid genera and species within the seven latitudinal zones are also shown. All physico-chemical data are presented as mean (± SEM) or median values within each zone.

	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	Zone 7
Sampling locations	11	12	11	15	15	14	3
EPHEMEROPTERA							
Individuals	3730	2922	3046	3252	2942	1895	234
Species	34	29	33	31	24	24	9
Genera	18	17	18	12	11	11	7
Families	8	8	8	7	6	7	4
Leptophlebiidae, species	26	21	24	22	16	15	6
Leptophlebiidae, genera	11	10	11	6	5	5	4
PHYSICO-CHEMICAL DATA							
Altitudinal range (m ASL)	10–210	10–550	75–775	20–975	25–1000	15–405	5–45
Altitude (m ASL)	71 ± 16	169 ± 47.2	357 ± 75	259 ± 75	379 ± 79	184 ± 34	20 ± 10
Air temperature* (4 hr, °C)	14.6 ± 0.5	14.5 ± 0.7	13.9 ± 0.7	14.9 ± 0.5	13.3 ± 0.6	12.4 ± 0.9	12.7 ± 1.6
Water temperature (°C)	16.3 ± 0.5	15.5 ± 0.8	14.1 ± 0.6	14.5 ± 0.5	12.9 ± 0.5	12.4 ± 0.9	11.3 ± 0.6
Dissolved oxygen (mg/L)	9.3 ± 0.4	9.6 ± 0.2	9.9 ± 0.2	10.9 ± 0.2	10.4 ± 0.5	10.4 ± 0.3	10.8 ± 0.1
Conductivity (μS_{25}/cm)	125 ± 8	113 ± 21	116 ± 25	77 ± 7	71 ± 14	90 ± 14	147 ± 20
pH	7.5 ± 0.1	7.6 ± 0.1	7.7 ± 0.1	7.3 ± 0.1	7.1 ± 0.2	7.2 ± 0.1	6.4 ± 0.3
Velocity estimate (ms^{-1})	0.2–0.4	0.2–0.4	0.2–0.4	0.4–0.6	0.2–0.4	0.2–0.4	0.2–0.4
Wetted width (m)	6.05 ± 1.5	5.11 ± 1.3	3.91 ± 0.5	6.71 ± 1.3	5.73 ± 0.7	5.56 ± 1.0	2.97 ± 0.6
Median wetted width (m)	3.01	3.88	3.86	5.38	4.58	4.86	3.40
Pfankuch Index Score	65 ± 6	58 ± 4	83 ± 7	65 ± 4	65 ± 3	71 ± 4	65 ± 4
Canopy cover (%)	69 ± 10	69 ± 10	60 ± 6	33 ± 9	29 ± 8	55 ± 9	80 ± 5
Substrate size (cm)	10.4 ± 2.5	17.6 ± 3.0	20.0 ± 6.9	16.5 ± 1.8	21.3 ± 3.4	12.4 ± 1.3	8.7 ± 3.9
Median substrate size (cm)	7.0	15.9	13.5	18.1	14.1	11.6	5.6

* Air temperature data are mean values over the four hours that light trapping was undertaken.

were acidic. The Pfankuch index indicated that stream stability was generally good in all zones and a high degree of uniformity in the mean size of stream bed material was found throughout the country. Species richness ranged from 24 in Russell Forest in Northland to three below the Rob Roy Glacier in Mount Aspiring National Park. Five of the six highest species richness counts were recorded at locations in Northland (mean species richness 22; range 20–24; *n* = 5), whereas the lowest six species richness counts were all in the lower South Island, including two locations on Stewart Island (mean species richness 4; range 3–4; *n* = 6).Regional level diversity ranged from 34 species in latitudinal Zone 1 to nine species in latitudinal Zone 7. A clear pattern of decreasing taxa richness across the latitudinal gradient (from north to south) was observed at the familial, generic and species levels (Table 4.6; see also latitude and altitude sections below) and a significant decrease in species richness was found with increasing latitude, i.e. north to south when the location data were plotted (R^2 = 0.67; $F_{1, 79}$ = 161; P < 0.001, Figure 4.8).

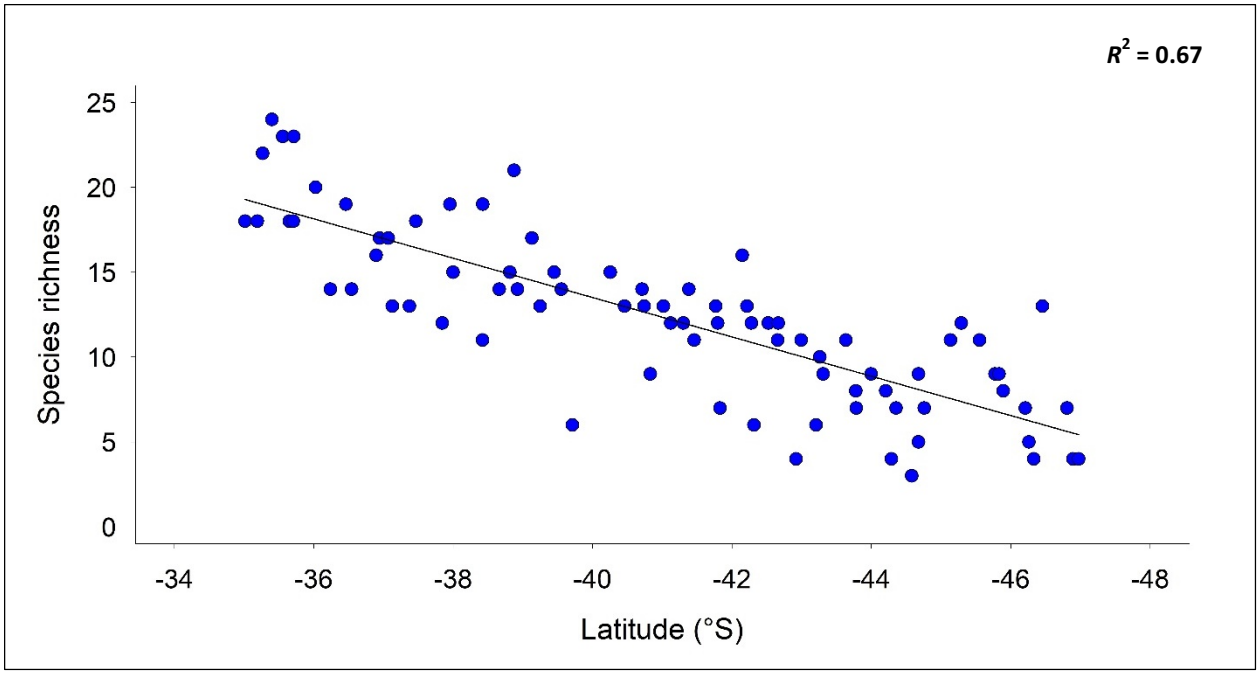


Figure 4.8 Species richness per location across the latitudinal gradient.

Of the almost 14,000 individuals taken in light traps, 39% were imagos and 61% subimagos; 63% of imagos were female, whereas the sex ratio of subimagos was approximately even (54% male, 46% female). Three gynandromorph imagos, showing both male and female characters (bilaterally asymmetrical forms), were also collected (0.0002% of light-trapped specimens). The most commonly encountered species nationwide (light trapping and benthic sampling combined) were *Coloburiscus humeralis*, *Ameletopsis perscitus* and *Neozephlebia scita* (Table 4.7). In contrast, nine species were only recorded once, and one was recorded twice. In the North Island, 37 species were recorded, the most commonly encountered being *Coloburiscus humeralis*, *Acanthophlebia cruentata* and *Zephlebia dentata* whereas four species were encountered at one location each (Table 4.7). No *Deleatidium* featured in the North Island top ten. In the South Island, 33 species were recorded with the most commonly recorded

being *Deleatidium* (*D.*) *myzobranchia* and *Coloburiscus humeralis* (recorded at 84% and 80% of stream locations, respectively). In contrast, seven species were found at one location each. Eighteen species were shared across both the North and South Islands (Table 4.8), and while some species were commonly encountered on both islands (*Coloburiscus humeralis*, *Ameletopsis perscitus*, *Neozephlebia scita*), others were more common on either the North Island (*Zephlebia versicolor* and *Austroclima sepia*) or the South Island (*Deleatidium* (*D*.) *myzobranchia* and *Deleatidium* (*D*.) *fumosum*).

A nonmetric multidimensional scaling ordination based on presence–absence mayfly data obtained for all sampling localities throughout New Zealand clearly separated almost all North Island localities from South Island localities (Figure 4.9). The South Island symbol shown to the right of the North Island cluster in the ordination diagram represents the Invercargill locality where only four species were recorded, all of which also occur in the North Island. The two North Island locality symbols at the right of the South Island cluster represent Mt Taranaki and Sentry Box (Ruahine Ranges) localities. Both these locations were at high-altitude (~600 m ASL) in the central North Island and their faunas included *Deleatidium* (*D*.) *myzobranchia* and *D*. (*D*.) *autumnale*, species that were more commonly found in the South Island. SIMPER indicated that the mayfly species contributing most strongly to the separation of localities in the two islands were all widely distributed in the North Island and absent or rarely found in the South Island (Table 4.9).

Table 4.7 The ten most common (upper) and uncommon (lower) species found in the nationwide survey of New Zealand (81 localities, 243 sites), North Island (36 localities, 108 sites) and South + Stewart Islands (45 localities, 135 sites).

New Zealand		North Island		South and Stewart Islands	
Species (52 recorded)	**Locations (%)**	**Species (37 recorded)**	**Locations (%)**	**Species (33 recorded)**	**Locations (%)**
Coloburiscus humeralis	88	*Coloburiscus humeralis*	97	*Deleatidium* (*D.*) *myzobranchia*	84
Ameletopsis perscitus	70	*Acanthophlebia cruentata*	92	*Coloburiscus humeralis*	80
Neozephlebia scita	69	*Zephlebia dentata*	92	*Deleatidium* (*D.*) *fumosum*	73
Deleatidium (*D.*) *myzobranchia*	62	*Zephlebia versicolor*	89	*Neozephlebia scita*	64
Deleatidium (*D.*) *fumosum*	60	*Austroclima sepia*	83	*Ameletopsis perscitus*	62
Nesameletus ornatus	57	*Ameletopsis perscitus*	81	*Nesameletus ornatus*	51
Zephlebia spectabilis	51	*Zephlebia borealis*	78	*Deleatidium* (*D.*) *vernale*	47
Austroclima sepia	47	*Neozephlebia scita*	75	*Austroclima jollyae*	44
Zephlebia versicolor	46	*Ichthybotus hudsoni*	72	*Deleatidium* (*D.*) *autumnale*	40
Acanthophlebia cruentata	41	*Zephlebia spectabilis*	69	*Oniscigaster distans*	38
Zephlebia dentata	41	–		–	
Rallidens platydontis	2	*Zephlebia nebulosa*	19	*Deleatidium* (*D.*) "rakiura"	7
Deleatidium (*D.*) "lyellensis"	1	*Austroclima jollyae*	14	*Nesameletus* "fiordlandi"	7
Deleatidium (*D.*) *acerbum*	1	*Deleatidium* (*D.*) *autumnale*	11	*Rallidens platydontis*	4
Deleatidium (*D.*) *magnum*	1	*Deleatidium* (*D.*) *vernale*	11	*Deleatidium* (*D.*) "lyellensis"	2
Deleatidium (*D.*) *cornutum*	1	*Oniscigaster distans*	11	*Deleatidium* (*D.*) *acerbum*	2
Deleatidium (*D.*) *patricki*	1	*Tepakia caligata*	11	*Deleatidium* (*D.*) *cerinum*	2
Mauiulus aquilus	1	*Deleatidium* (*D.*) *magnum*	3	*Deleatidium* (*D.*) *cornutum*	2
Nesameletus murihiku	1	*Mauiulus aquilus*	3	*Deleatidium* (*D.*) *patricki*	2
Zephlebia aff. *pirongia* sp. 1	1	*Zephlebia* aff. *pirongia* sp. 1	3	*Nesameletus murihiku*	2
Zephlebia pirongia	1	*Zephlebia pirongia*	3	*Oniscigaster wakefieldi*	2

The nationwide survey has markedly extended the known ranges of a number of species, notably *Arachnocolus phillipsi*, *Austroclima sepia*, *Austronella planulata*, *Deleatidium* (*D*.) *lillii*, *Isothraulus abditus*, *Mauiulus luma*, *Oniscigaster distans*, *O*. *wakefieldi*, *Rallidens mcfarlanei*, *Siphlaenigma janae*, *Tepakia caligata*, *Zephlebia borealis*, *Z*. *inconspicua* and *Z*. *nebulosa* (see distribution tables and map figures presented at the end of the chapter). Knowledge of the distribution of most other species has also been increased by filling 'distribution gaps'. Several changes to currently understood species distributions are also proposed based on specimens collected during the nationwide survey, and the re-examination of museum collection material. These include the removal of *Zephlebia dentata*, *Z*. *inconspicua* and *Z*. *pirongia* from the South Island fauna, and the re-assignment of most South Island material attributed to *Z. dentata* and *Z*. *nebulosa* to a tentative new species, *Zephlebia* sp. 1 (see Appendix 2).

Table 4.8 The percentage of locations at which all 18 mayfly species found in both the North and South Islands (36 and 45 localities, respectively) were recorded in the nationwide survey. Percentages are listed in decreasing order as found in the North Island.

Species	North Island Locations (%)	South Island Locations (%)
Coloburiscus humeralis	97	80
Zephlebia versicolor	89	11
Austroclima sepia	83	18
Ameletopsis perscitus	81	62
Neozephlebia scita	75	64
Zephlebia spectabilis	69	36
Nesameletus ornatus	64	51
Deleatidium (*D*.) *lillii*	56	20
Deleatidium (*D*.) *fumosum*	44	73
Deleatidium (*D*.) *cerinum*	36	2
Deleatidium (*D*.) *myzobranchia*	33	84
Nesameletus flavitinctus	33	20
Oniscigaster wakefieldi	28	2
Atalophlebioides cromwelli	25	20
Austroclima jollyae	14	44
Deleatidium (*D*.) *autumnale*	11	40
Deleatidium (*D*.) *vernale*	11	47
Oniscigaster distans	11	38

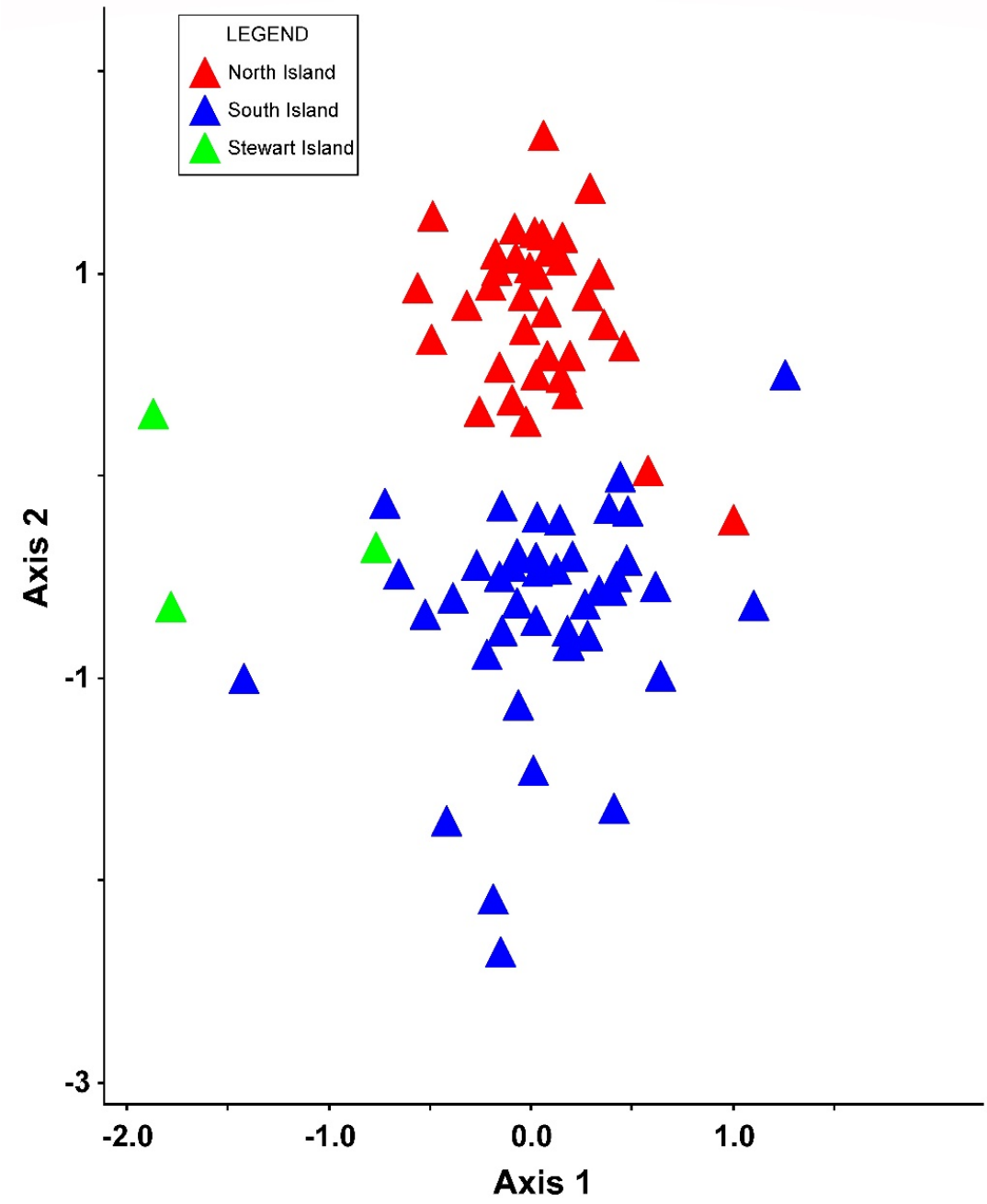


Figure 4.9 NMS ordination (presence–absence data) of mayfly assemblages obtained at 81 localities throughout New Zealand. Stress = 15.3; *P* = 0.002.

Table 4.9 The six mayfly species contributing most strongly to the separation of North and South Island localities in the NMS ordination. Percentage abundance refers to the proportion of localities at which the species were present.

	Mean	Percentage abundance	
Species	**dissimilarity**	**North Island**	**South Island**
Acanthophlebia cruentata	3.734	91.7	0
Zephlebia dentata	3.655	91.7	0
Zephlebia versicolor	3.251	88.9	11.9
Zephlebia borealis	3.029	77.8	0
Austroclima sepia	2.955	83.3	14.3
Ichthybotus hudsoni	2.791	72.2	0

Effect of latitude and altitude on species richness

Latitude and altitude can be expected to have comparable effects on faunal elements because environmental temperature decreases as both variables increase. When species richness at the 78 sampled localities on the North and South islands (but not Stewart Island) were included as the dependent variable in a multiple regression with latitude and altitude as independent variables a highly significant result was obtained (R^2 = 0.68; $F_{2, 75}$ = 80; P < 0.001). Individual R^2 values for the two variables were significant but indicated a stronger effect of latitude on species richness (latitude R^2 = 0.64, P < 0.001; altitude R^2 = 0.13, P < 0.01). The relationships with the two variables are shown graphically within the text below.

Latitude

Numbers of mayfly species found in Zones 1–6 (the latitudinal gradient) ranged from 34 to 24 (Table 4.6; Figure 4.10a). Largest numbers of species were found in the northernmost Zone 1 and fewest in Zones 5 and 6 (24 in each). Even fewer species (9) were found in Zone 7 (Stewart Island) but as only three locations could be sampled on the island, direct comparisons of species richness with the other zones are not warranted. Zone 7 is therefore presented in some figures for completeness, but not considered in statistical analyses. Margalef's index (Figure 4.10a) showed a similar decline in species richness with increasing latitude, while Whittaker's species turnover increased with distance from most zones (Table 4.10) and is depicted with reference to Zone 1 in Figure 4.10b.

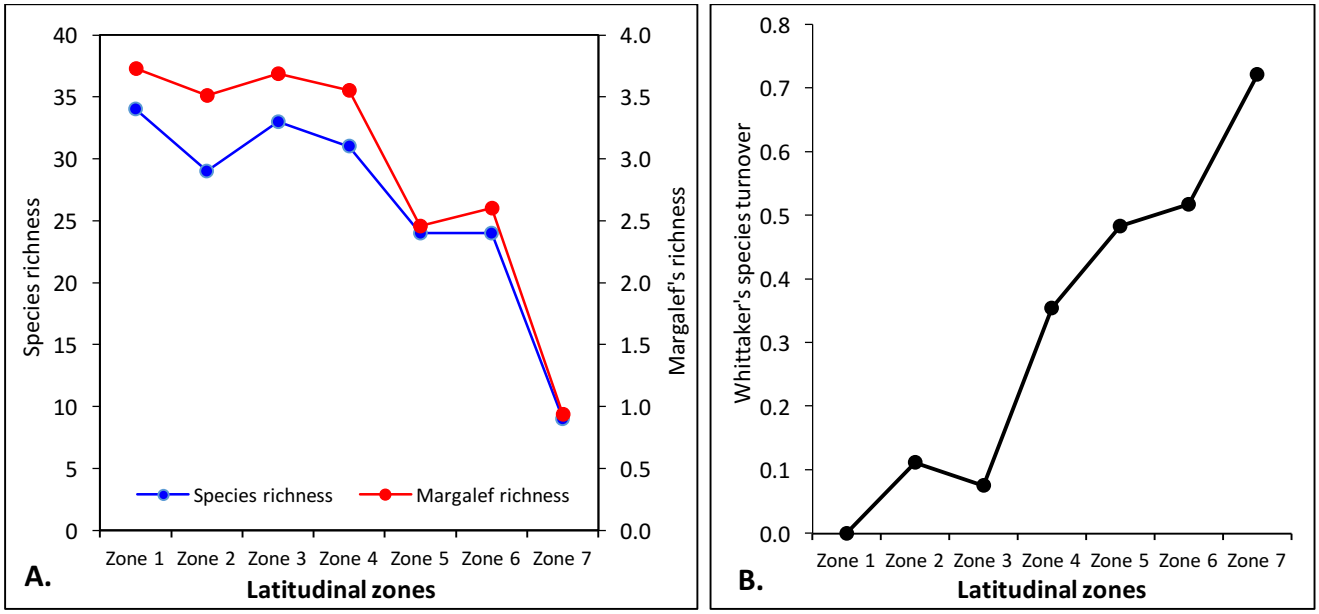


Figure 4.10 Measures of mayfly diversity with latitude across New Zealand. **A.** Total species richness (combined benthic sampling and light trapping data) and Margalef's richness (light trapping data only). **B.** Change in community composition, as measured by Whittaker's species turnover for pairwise comparisons with Zone 1 data. For complete pairwise data of Whittaker's species turnover see Table 4.10.

Table 4.10 Change in mayfly species composition among latitudinal zones as indicated by Whittaker's species turnover.

Zones	1	2	3	4	5	6
1	0.000	0.111	0.075	0.354	0.483	0.517
2		0.000	0.129	0.367	0.509	0.547
3			0.000	0.313	0.439	0.474
4				0.000	0.200	0.236
5					0.000	0.167
6						0.000

Similarity of the mayfly fauna among latitudinal zones, assessed with Sørensen's index, ranged from 0.925 to 0.453, and with minor exceptions declined with distance between zones (Table 4.11). Similarity of the large leptophlebiid component of the fauna mirrored that of the total mayfly fauna and ranged from 0.920 to 0.444 (Table 4.12). Latitudinal differences in the mayfly fauna among zones, with reference to the northernmost (Zone 1) and southernmost (Zone 6) zones are shown in Figure 4.11. It can be seen that similarity declined strongly from north to south and south to north, respectively, and that the relationship was very strong (Pearson's correlation coefficient; $P < 0.01$).

Table 4.11 Similarity of the mayfly fauna among latitudinal zones, assessed with the Sørensen's index.

Zones	1	2	3	4	5	6
1	1.000	0.889	0.925	0.646	0.517	0.483
2		1.000	0.871	0.633	0.491	0.453
3			1.000	0.688	0.561	0.526
4				1.000	0.800	0.764
5					1.000	0.833
6						1.000

Table 4.12 Similarity of the Leptophlebiidae fauna among latitudinal zones, assessed with the Sørensen's index.

Zones	1	2	3	4	5	6
1	1.000	0.851	0.920	0.667	0.524	0.488
2		1.000	0.844	0.651	0.486	0.444
3			1.000	0.696	0.550	0.513
4				1.000	0.789	0.703
5					1.000	0.839
6						1.000

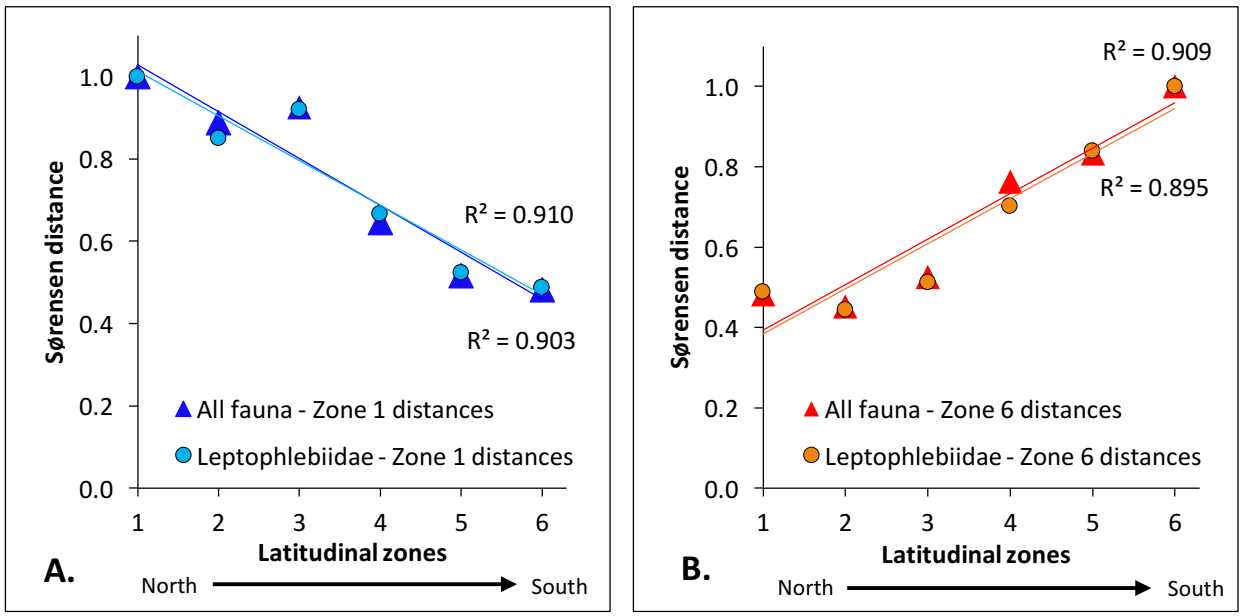


Figure 4.11 Similarity of the mayfly faunas among latitudinal zones, assessed with the Sørensen's index. For complete pairwise data see Tables 4.11 and 4.12 above. **A.** Similarity of Zone 1 mayfly fauna with all other zones. **B.** Similarity of Zone 6 mayfly fauna with all other zones.

Altitude

Seventy-eight locations from the nationwide survey (excluding three Stewart Island locations) were placed into six altitudinal bands for comparisons of species richness and faunal similarity with altitude. Altitudinal bands were selected to incorporate approximately equal numbers of locations per band. Total numbers of mayfly species recorded in the six bands ranged from 32–43 and numbers of the Leptophlebiidae fauna ranged from 23–31 (Figure 4.12). Both taxon groups showed little variation in species richness among bands 1–4 but declined above 330 m a.s.l.

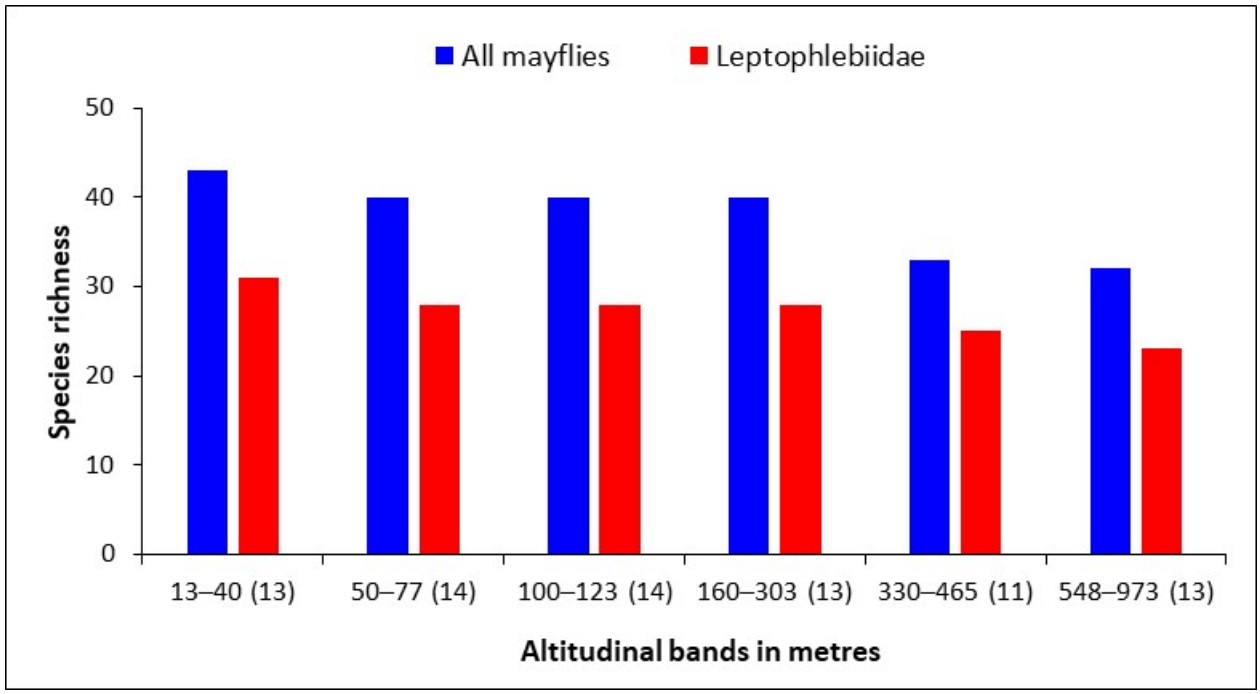


Figure 4.12 Numbers of mayfly species and Leptophlebiidae species found in six bands of altitude throughout New Zealand. Values in parentheses indicate the number of locations included per band.

As indicated above, multiple regression analysis showed that species richness of the mayfly fauna at sampling localities throughout New Zealand was significantly influenced by altitude (R^2 = 0.13, P < 0.01), although not as strongly as by latitude (R^2 = 0.64, P < 0.001). Thus, although fewer species were found at higher altitudes, species richness varied considerably along the elevation gradient (Figure 4.13).

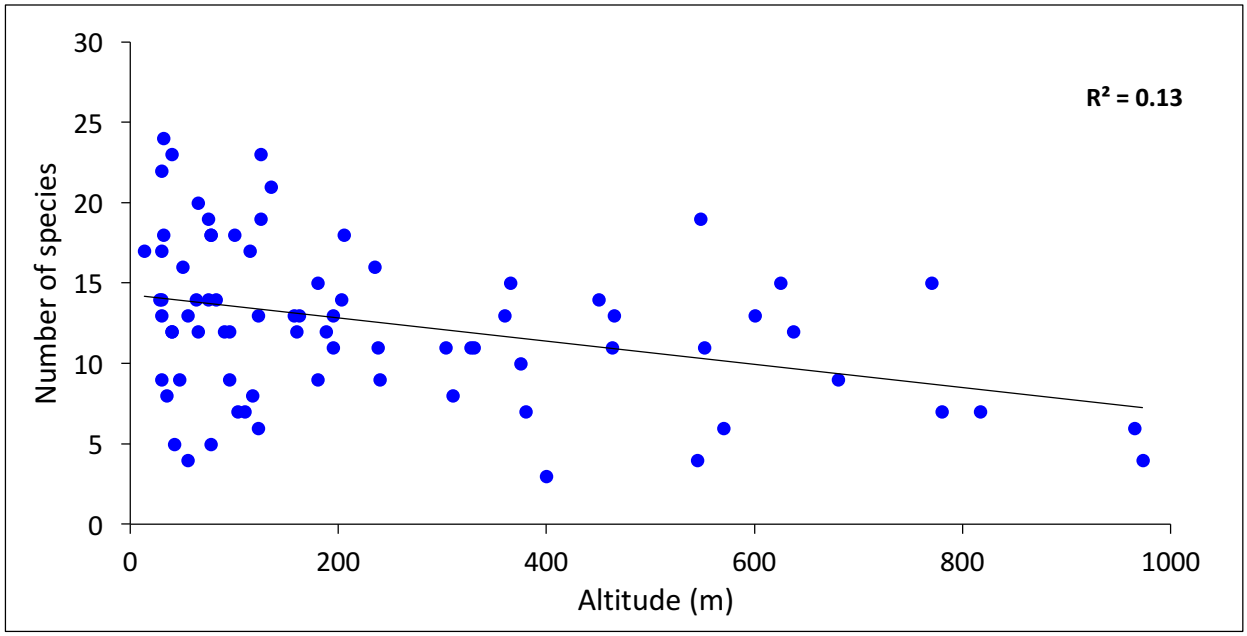


Figure 4.13 Species richness per location across an altitudinal gradient based on the nationwide survey (n = 78, excludes Stewart Island locations).

Similarity of the mayfly fauna among six altitudinal bands, assessed with the Sørensen's index, ranged from 0.950 to 0.740, and showed a shallow decline with distance between altitudes (Table 4.13). Similarity of the leptophlebiid component of the fauna mirrored that of the total mayfly fauna and ranged from 0.964 to 0.745 (Table 4.14). The extent of differences among altitudinal bands are shown with reference to the lowermost (13–40 m) and uppermost (548–973 m) bands in Figure 4.14. Although faunal similarity declined with altitude the differences found were not as pronounced as those seen in relation to latitude.

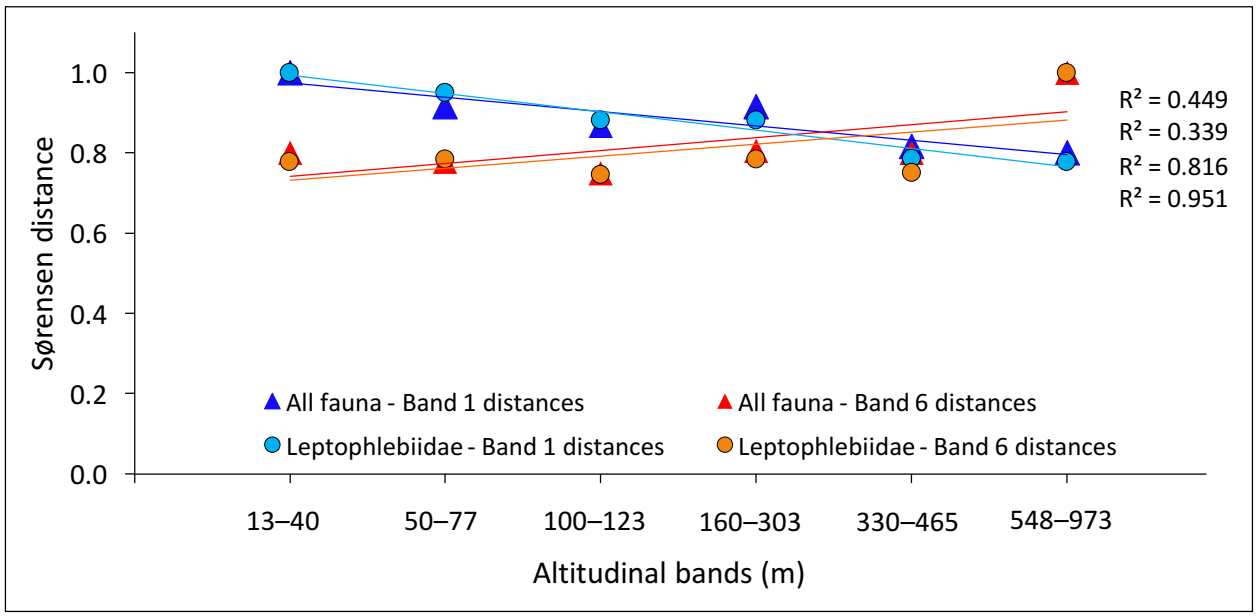


Figure 4.14 Similarity of total mayfly and Leptophlebiidae faunas between altitudinal bands, assessed with Sørensen's index. Data presented are similarity of the lowest and highest bands with all other altitudes. For complete pairwise data see Tables 4.13 and 4.14.

Table 4.13 Similarity of the mayfly fauna among altitudinal bands, assessed with the Sørensen's index.

Bands	1	2	3	4	5	6
1	1.000	0.916	0.867	0.916	0.816	0.800
2		1.000	0.925	0.950	0.822	0.778
3			1.000	0.875	0.740	0.750
4				1.000	0.822	0.806
5					1.000	0.800
6						1.000

Table 4.14 Similarity of the Leptophlebiidae fauna among altitudinal bands, assessed with the Sørensen's index.

Bands	1	2	3	4	5	6
1	1.000	0.949	0.881	0.881	0.786	0.778
2		1.000	0.893	0.964	0.830	0.784
3			1.000	0.857	0.792	0.745
4				1.000	0.830	0.784
5					1.000	0.750
6						1.000

Distribution of mayflies on Mt Taranaki in relation to altitude

Data obtained during the nationwide survey indicated that species richness of mayflies declined significantly, although weakly, with altitude. Further, it indicated that size of the mayfly *Coloburiscus humeralis* increased along the elevational gradient (see 'Distribution–size relationships of *Coloburiscus humeralis*' section below). To further investigate whether altitude affected species richness and mayfly size a case study was undertaken along an altitudinal gradient on Mt Taranaki, a volcanic cone in the North Island. The streams on the mountain flow down through native forest, and therefore provide near-natural ecosystems, which are also protected conservation estate. Many of the streams are accessible

through a network of walking tracks and arterial roads, although it was not logistically possible to select sampling locations on a single stream.

Seven locations at altitudes ranging from 120 m to 1100 m a.s.l. were sampled on two occasions by light-trapping and benthic sampling (see Methods for details). A total of twenty-one mayfly species were collected. Species richness ranged from 5 to 15 species per altitudinal location and declined significantly with increasing altitude (R^2 = 0.79; $F_{1, 5}$ = 18.7; P < 0.01) (Figure 4.15a). Most species collected on Mount Taranaki at or below the 730 m location were species common to the North Island. However, at the 730 m, 880 m and 1100 m locations a number of species common to the South Island (*Deleatidium* (*D.*) *autumnale*, *D.* (*D.*) *myzobranchia*, *Oniscigaster distans*) were also collected. In addition, between 730 m and 1100 m, 64 specimens of *Deleatidium* (*D.*) *magnum* were collected, an uncommon species known only from four confirmed locations at high elevations in the central North Island (but also several unconfirmed South Island records).

A significant increase in forewing length of male and female *C. humeralis* was observed with increasing altitude (R^2 = 0.78; $F_{2, 163}$ = 288; P < 0.001) (Figure 4.15b). The largest *C. humeralis* males and females were taken at 600 m a.s.l. and the smallest at 120 m a.s.l. Few individuals were taken at 730 m a.s.l. and none at the two highest elevation sites.

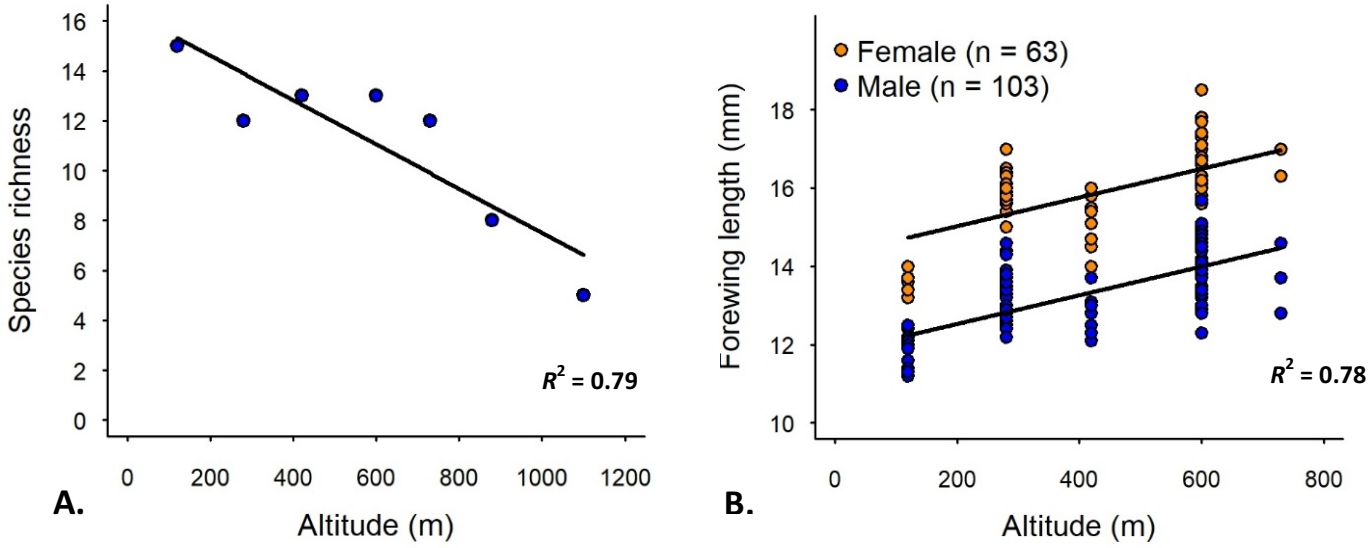


Figure 4.15 Relationships between species richness and size of *Coloburiscus humeralis* along an altitudinal gradient on Mt Taranaki. Data from the two surveys combined. **A.** Mayfly species richness. **B.** *Coloburiscus humeralis* forewing length. R^2 = combined male + female relationship. Note that *C. humeralis* was not taken at the two uppermost locations (880 m and 1100 m).

Distribution–size relationships of *Coloburiscus humeralis*

Forewing lengths of 741 male and 577 female *C. humeralis* imagos and subimagos collected from throughout New Zealand were measured. Males ranged in size from 9.5 to 16.3 mm and females 10.5 to 18.2 mm. Mean forewing lengths of males were significantly smaller than females (*t*-test: t =7.6, P <

0.001). Much of the variability in size of males and females was associated with the altitude of collecting localities with those well above the regression line being from high altitudes and many of the smaller mayflies being from near sea level (Figure 4.16).

Results of a multiple regression analysis with forewing length as the dependent variable and latitude and altitude as independent variables showed a highly significant increase in size with latitude and altitude (R^2 = 0.36; $F_{2, 103}$ = 28.6; P < 0.001). There was no interaction effect of sex with latitude (P = 0.08) and both males and females combined showed a significant increase in forewing length with latitude (R^2 = 0.68; $F_{2, 103}$ = 108; P < 0.001), individuals in the north being smaller than those in the south (Figure 4.16).

There was no interaction effect of sex with altitude (P = 0.86) and the relationship between altitude and forewing length of both sexes combined was also significant (R^2 = 0.46; $F_{2, 103}$ = 43; P < 0.001). However, the data showed considerable variation throughout the altitudinal range (Figure 4.17), likely due to other confounding factors. Because increases in latitude and altitude are correlated with declining environmental temperature, the results suggest that body size (forewing length) of *C. humeralis* is affected by temperature, perhaps by influencing the length of the nymphal life stage, and in colder waters, enabling the nymph to grow larger. Harding and Winterbourn (1993) calculated that the length of the life cycle of *C. humeralis* was about 18 months in a lake outlet stream and 27 months in a colder forest stream at the same altitude, which lends plausibility to this argument. Unfortunately, winged stages were not sampled in their study and the sizes attained by nymphs are not given.

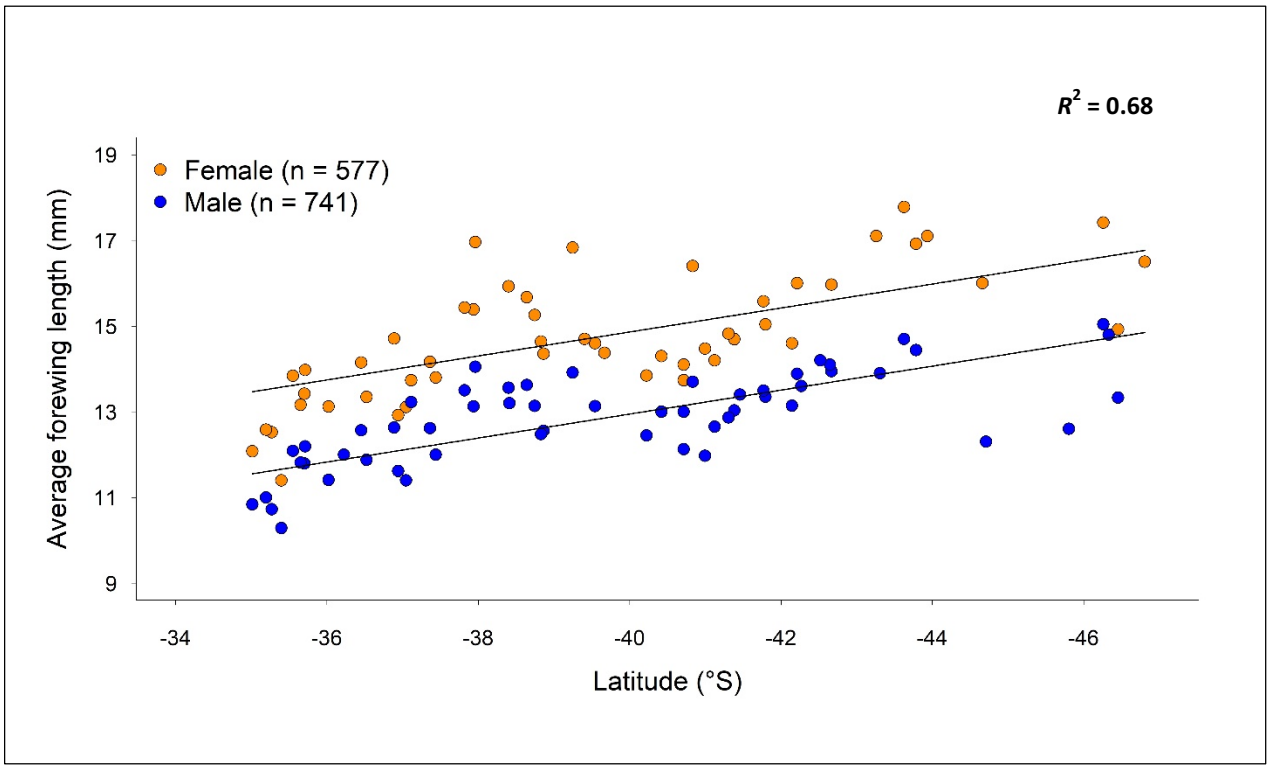


Figure 4.16 Mean forewing lengths of male and female *Coloburiscus humeralis* imagos and subimagos with latitude, taken from 71 localities throughout New Zealand. R^2 = combined male + female relationship.

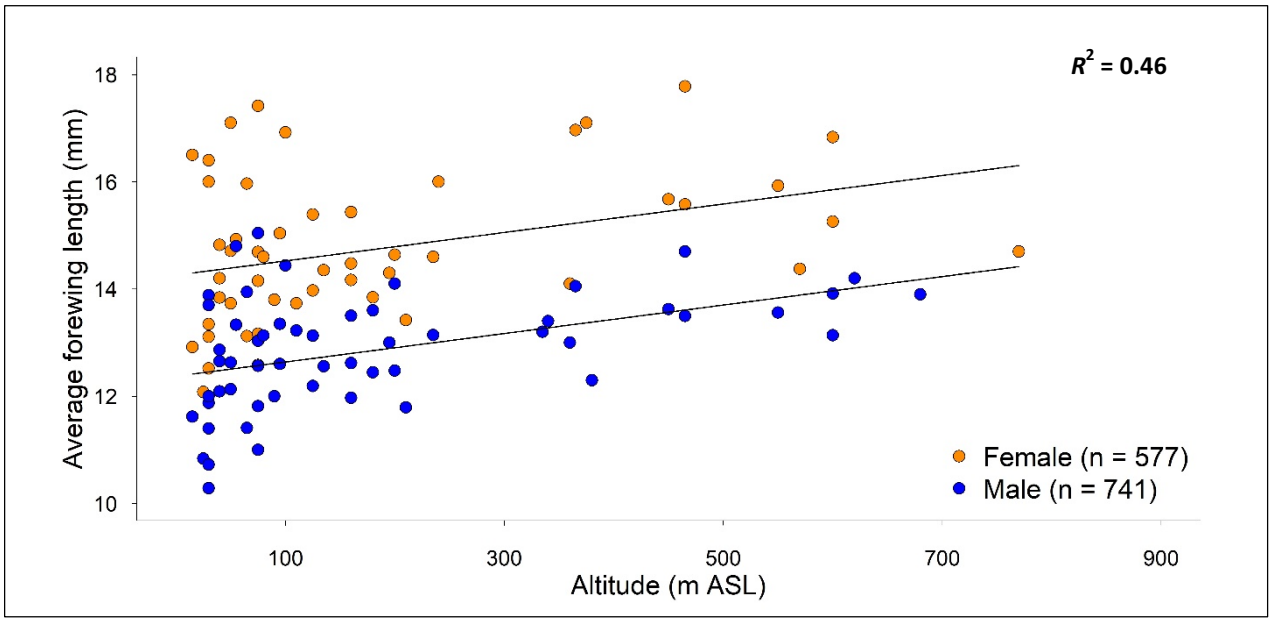


Figure 4.17 Mean forewing lengths of male and female *Coloburiscus humeralis* imagos and subimagos with altitude, taken from 71 localities throughout New Zealand. R^2 = combined male + female relationship.

Overview of mayfly distribution in New Zealand

In this section of the chapter data from my nationwide survey, historical records and published sources are combined to provide an overview of mayfly distribution in New Zealand. Representatives of five of the eight families are found in all three main islands of New Zealand (North, South and Stewart islands) and the other three families have representatives in the North and South Islands only i.e. not on Stewart Island (Table 4.15). Of the 20 mayfly genera, seven are found on North, South and Stewart Islands, seven are known from the North and South Islands, but have not been recorded on Stewart Island, and six genera are restricted to the North Island. No genera are endemic to the South Island or Stewart Island but one monospecific genus, *Cryophlebia* (Leptophlebiidae), is endemic to the sub-Antarctic Auckland Islands. Eleven species are known from all three main islands of New Zealand, 23 are shared by the North and South islands, 15 are known from the North Island only and 16 from the South Island only. Overall, 41 species have been recorded in the South Island, 38 in the North Island, 14 on Stewart Island, and one on the Auckland Islands (Table 4.16). No mayfly species have been found on the Chatham Islands or other sub-Antarctic islands of the New Zealand.

New Zealand's mayfly diversity is greatest in the north and west of the North Island and the northwest of the South Island (Figure 4.18). The Leptophlebiidae is by far the most diverse family with 43 identified species in 13 genera. It is also notable that three families are represented by single species and three others have a pair of species each (Table 4.15). Total species richness, and species richness of the most speciose family, Leptophlebiidae, are similar on the North and South Islands (Table 4.16). In contrast, members of the two most speciose genera are not evenly distributed, with *Deleatidium* having greater species richness and stronger levels of endemism in the South Island and *Zephlebia* having greater species richness in the North Island (Table 4.17).

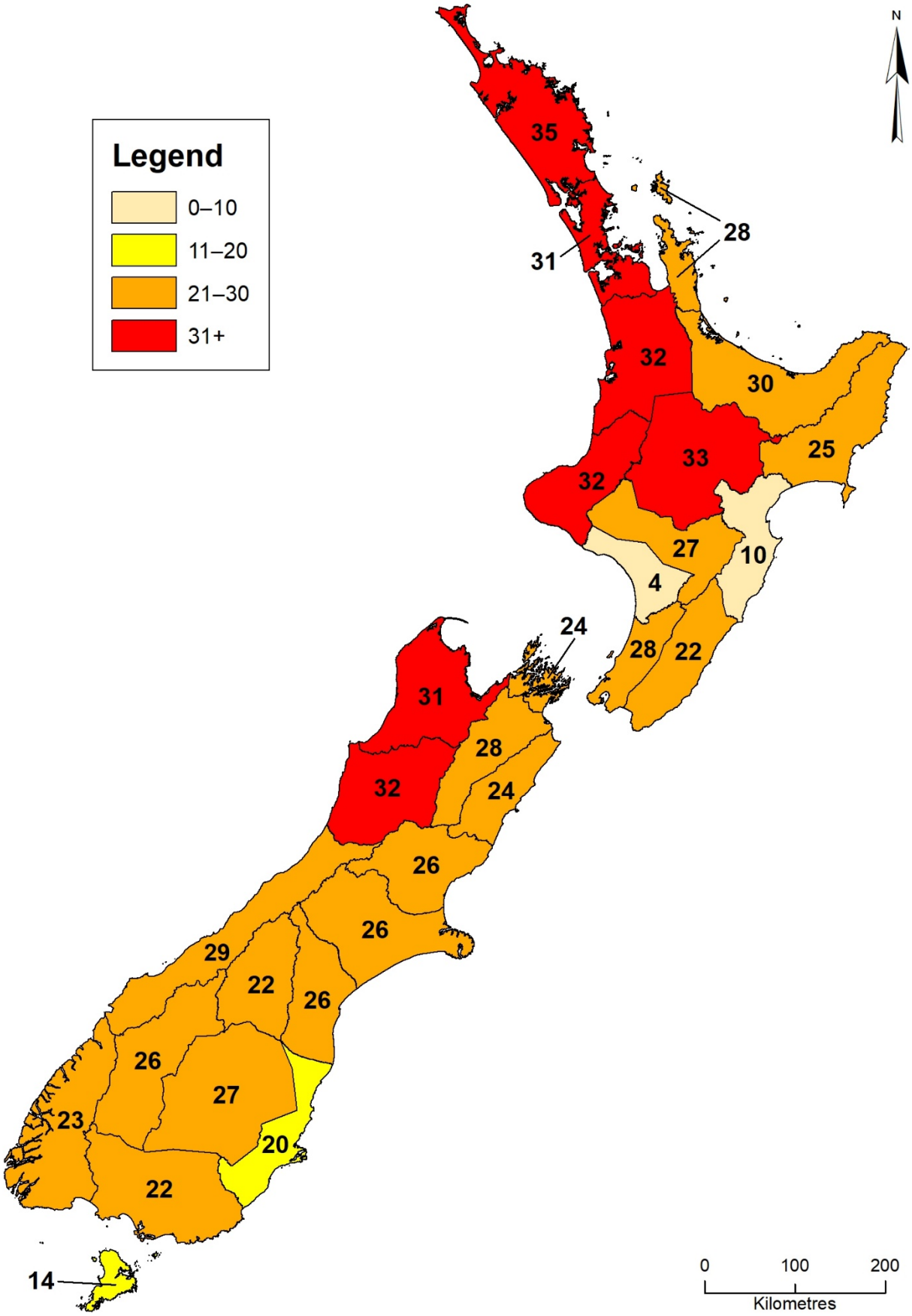


Figure 4.18 Recognised species richness of New Zealand Ephemeroptera within the geographical areas defined by Crosby et al. (1998).

Table 4.15 Summary of the taxonomic composition of the New Zealand mayfly fauna (including tentative species) recorded historically and in my nationwide survey. N = North Island, S = South Island, St = Stewart Island, A = Auckland Islands.

Families	Distribution of families	Number of genera	Number of species
Ameletopsidae	N, S, St	1	1
Coloburiscidae	N, S, St	1	1
Ichthybotidae	N, S	1	2
Leptophlebiidae	N, S, St, A	13	43
Nesameletidae	N, S, St	1	6
Oniscigastridae	N, S, St	1	2
Rallidentidae	N, S	1	2
Siphlaenigmatidae	N, S	1	1

Table 4.16 Numbers of *Deleatidium*, *Zephlebia*, Leptophlebiidae and all mayfly species recorded from the three main islands of New Zealand (includes tentative species, historical records and nationwide survey records).

Taxa	North Island	South Island	Stewart Island	All of New Zealand
Deleatidium	8	19	5	20
Zephlebia	9	4	1	10
Leptophlebiidae	29	28	9	43
All mayflies	38	41	14	60

Table 4.17 Numbers of species of *Deleatidium*, *Zephlebia* and other Leptophlebiidae recorded in different parts of New Zealand (includes tentative species, historical records and nationwide survey records).

Taxa	North, South & Stewart Is.	North & South Is. only	North Is. only	South Is. only	Stewart Is. only
Deleatidium	4	4	0	10	1
Zephlebia	1	2	6	1	0
Other Leptophlebiidae	3	2	7	0	0
All mayflies	11	12	15	16	1

Distribution patterns of individual species

Distribution patterns of all New Zealand species of Ephemeroptera are provided in the distribution tables and map figures at the end of the chapter. Some of the distribution data differ in from those shown on maps provided by other workers (i.e. Hitchings 2001, 2008; Hitchings et al. 2015) due to re-identification of some material. The five principal distribution patterns found are discussed below. Species acknowledged as widespread are listed with ^{W} and those as sparse with ^{Sp}. Species can also be widely distributed, but sparse over all or part their ranges ($^{W, Sp}$) or conversely, restricted to a particular area within that distribution (^{R}).

Species occurring on North, South and Stewart Islands

Eleven species, belonging to seven genera and four families are found in the three main islands. All are fairly widespread species, although *Oniscigaster distans* and *Deleatidium* (*D.*) *myzobranchia* appear to be sparsely distributed north of Taupo.

Species in this group are: *Ameletopsis perscitus* [W], *Coloburiscus humeralis* [W], *Austroclima jollyae* [W], *Austroclima sepia* [W], *Deleatidium* (*D.*) *cerinum* [W], *Deleatidium* (*D.*) *fumosum* [W], *Deleatidium* (*D.*) *lillii* [W], *Deleatidium* (*D.*) *myzobranchia* [W, Sp], *Neozephlebia scita* [W], *Zephlebia spectabilis* [W], *Oniscigaster distans* [W, Sp].

Species occurring on North and South Islands only

Twelve species, belonging to seven genera and four families are found on both North and South islands. *Deleatidium* (*D.*) *autumnale* and *Deleatidium* (*D.*) *vernale* are widespread in the South Island but occur sparsely in the upper North Island, whereas *Nesameletus flavitinctus* is widespread on both islands, but currently not recorded from Southland. In contrast, *Deleatidium* (*D.*) *magnum* is definitely known only from Mount Taranaki and the volcanic plateau in the North Island, and has been reported in the South Island, although all South Island records of *D.* (*D.*) *magnum* need validating.

Species in this group are: *Atalophlebioides cromwelli* [W], *Deleatidium* (*D.*) *angustum* [W], *Deleatidium* (*D.*) *autumnale* [W, Sp], *Deleatidium* (*D.*) *magnum* [R, Sp], *Deleatidium* (*D.*) *vernale* [W, Sp], *Mauiulus luma* [W, Sp], *Nesameletus flavitinctus* [W], *Nesameletus ornatus* [W], *Oniscigaster wakefieldi* [W], *Siphlaenigma janae* [W, Sp], *Zephlebia nebulosa* [W, Sp], *Zephlebia versicolor* [W].

It is worth noting that four of these species (*Mauiulus luma* [W, Sp], *Siphlaenigma janae* [W, Sp], *Zephlebia nebulosa* [W, Sp] and *Zephlebia versicolor* [W]) are known only from the North Island and the northwest of the South Island and therefore represent a subgroup. Three other species (*Zephlebia dentata, Zephlebia inconspicua*, and *Zephlebia pirongia*) were also considered to have this distribution pattern by Hitchings et al. (2015) but my research indicates that South Island material previously identified as belonging to these species was misidentified. All species in this group are widespread in the North Island, but three are also sparsely distributed below the volcanic plateau.

Species endemic to the North Island

Fifteen species belonging to ten genera and three families appear to be endemic to the North Island. *Deleatidium* (*D.*) *magnum* and *D.* (*D.*) *angustum*, currently recognised from both the North and South Islands, may also belong in this group. About half the North Island species are widespread e.g. *Acanthophlebia cruentata*, *Ichthybotus hudsoni*, *Tepakia caligata*, *Zephlebia borealis*, *Zephlebia dentata*. *Austronella planulata* and *Mauiulus aquilus* are also widespread but appear to be sparsely distributed. *Rallidens mcfarlanei* is also widespread in the North Island and two South Island records of its presence in the South Island need validation. Furthermore, *R. mcfarlanei*, as currently recognised, may actually

comprise two species with non-overlapping distributions: *R. mcfarlanei* in Northland and Auckland and an undescribed species further south (= *R. deathi* in my research notes, see Appendix 2). Other North Island endemics are four species restricted to the upper North Island (*Arachnocolus phillipsi*, *Isothraulus abditus*, *Zephlebia inconspicua* and *Zephlebia tuberculata*) and three species with very restricted known distributions: *Zephlebia pirongia* in the Waikato, a tentative species *Zephlebia* aff. *pirongia* sp. 1 known only from Whangarei, and *Aupouriella pohei*, which has been found only near North Cape.

The fifteen species endemic to the North Island are therefore: *Acanthophlebia cruentata* [W], *Arachnocolus phillipsi* [R], *Aupouriella pohei* [R], *Austronella planulata* [W, Sp], *Ichthybotus hudsoni* [W], *Isothraulus abditus* [R], *Mauiulus aquilus* [W, Sp], *Tepakia caligata* [W], *Zephlebia borealis* [W], *Zephlebia dentata* [W], *Zephlebia inconspicua* [R], *Zephlebia pirongia* [R], *Zephlebia* aff. *pirongia* sp. 1 [R], *Zephlebia tuberculata* [R], *Rallidens mcfarlanei* [W].

Species endemic to the South Island (excluding Stewart Island)

Sixteen species, belonging to five genera and four families appear to be endemic to the South Island. Approximately 60% of these species belong to the genus *Deleatidium*. Only a few of this group are widespread, and they appear restricted to low elevations (*Ichthybotus bicolor*), higher elevations (*Deleatidium* (*D.*) *atricolor*, *D.* (*P.*) *cornutum, D.* (*P.*) *patricki* and *Nesameletus austrinus*), or have a poorly known distribution (*Rallidens platydontis*). *D.* (*D.*) *acerbum*, *D.* (*D.*) *kawatiri* and an undescribed species, *Zephlebia* sp. 1 are also South Island endemics with known distributions limited to the northwest of the South Island. The remaining nine species are known only from limited geographic regions and include *D.* (*D.*) *townsi* and *D.* (*D.*) *wardorum* from the northern three-quarters of the South Island, *Nesameletus vulcanus* from Banks Peninsula, the tentative species *Nesameletus* "fiordlandi" from Fiordland, *D.* (*P.*) *insolitum* from the inland Canterbury mountains, *D.* (*D.*) *branchiola* from the mountains surrounding Queenstown and an undescribed species *D.* (*D.*) "lyellensis" from the Lyell Range in Westland.

The sixteen species endemic to the South Island are therefore: *Deleatidium* (*D.*) *acerbum* [R], *Deleatidium* (*D.*) *atricolor* [W, R], *Deleatidium* (*D.*) *branchiola* [R], *Deleatidium* (*D.*) *kawatiri* [R], *Deleatidium* (*D.*) "lyellensis" [R], *Deleatidium* (*D.*) *townsi* [W, R], *Deleatidium* (*D.*) *wardorum* [W, R], *Deleatidium* (*P.*) *cornutum* [W, R], *Deleatidium* (*P.*) *insolitum* [R], *Deleatidium* (*P.*) *patricki* [W, R], *Ichthybotus bicolor* [W, R], *Nesameletus austrinus* [W, R], *Nesameletus* "fiordlandi" [R], *Nesameletus vulcanus* [R], *Rallidens platydontis* [W, Sp], *Zephlebia* sp. 1 [R].

Species endemic to oceanic islands

A single species, *Cryophlebia aucklandensis*, inhabits the sub-Antarctic Auckland Islands and is the only mayfly on an oceanic island in the New Zealand biogeographic region.

Discussion

Of the eight described species not recorded in my survey, two (*Coloburiscus tonnoiri* and *Oniscigaster intermedius*) are regarded as of doubtful validity (see Chapter 2) and two are only known from outside the surveyed area: *Aupouriella pohei*, which is known only from the very top of the North Island and *Cryophlebia aucklandensis*, which has been found only on the sub-Antarctic Auckland Islands. The four other unrecorded species, *Deleatidium* (*D.*) *branchiola*, *D.* (*D.*) *kawatiri, D.* (*Penniketellum*) *insolitum* and *Nesameletus vulcanus*, appear to have restricted ranges within the South Island, and were likely not encountered because of their rareness, the limited sampling done within their ranges, or due to chance. For example, *N. vulcanus* is known from only 12 streams on Banks Peninsula (South Island) and although I collected it from the sole survey location (Okuti River) on Banks Peninsula during a preliminary survey in 2012, was not recorded there during the nationwide survey in 2014.

Towns and Peters (1996) noted that the mayflies of New Zealand showed four broad distribution patterns: known only from the North Island, known only from the South Island, known only from sub-Antarctic islands, and found in both North and South islands. Since publication of their monograph on Leptophlebiidae numerous new species have been described, especially from the South Island, and I have been able to examine a large quantity of material in museum collections, not all of which had been identified correctly. My comprehensive light trapping and benthic survey across the length of the country, including Stewart Island, has greatly extended and clarified knowledge of the distributions of many mayfly species, and highlighted others of potential for further study. My findings confirm previous observations (particularly those of Towns & Peters 1996; Hitchings 2001; Hitchings et al. 2015) that mayfly diversity is particularly high in the north of the North Island and that diversity declines towards the south. My survey results also indicate that diversity is higher on the west than east of both main islands, a pattern that does not appear to have been noted before. The higher diversity in the north of the North Island is due to a number of genera and species, occurring only there, the most extreme example being *Aupouriella pohei*, which is known from a single stream close to North Cape.

Several workers have proposed networks of ecoregional and biogeographical zones as aids to understanding the distributions of plants and animals within New Zealand. The ecoregions proposed by Harding and Winterbourn (1997) were defined with particular reference to streams and small rivers and are based on climatic, geological and vegetation conditions. They successfully distinguish broad differences in whole benthic invertebrate communities, but are less useful for defining the distributions of mayfly species, many of which span a number of their ecoregions. Unsurprisingly, the exceptions are those species which appear to have very limited distributions.

It is generally agreed that the New Zealand mayflies have ancient Gondwanan origins and show phyletic affinities with other fragments of Gondwana including Australia and South America (Edmunds

1972; Towns & Peters 1980; Winterbourn 1980; Towns & Peters 1996). A number of the extant leptophlebiid genera also show affinities with New Caledonia (Edmunds 1972), which is also Gondwanan in origin and along with New Zealand is an exposed fragment of postulated continent of Zealandia (Luyendyk 1995).

Although general distribution patterns of aquatic insects, including Ephemeroptera (Towns & Peters 1996; Hitchings & Staniczek 2003), Plecoptera (McLellan 2006; McCulloch 2010) and Trichoptera (Henderson 1985; Heads 1997), have now been reasonably well documented in New Zealand, little attempt has been made to explain the distribution patterns. Hitchings et al. (2015) commented that Cook Strait is an effective barrier to mayfly migration and noted "ten species restricted to the north Island and 12 to the South Island". Hitchings and Staniczek (2003) suggested that *Nesameletus volcanus*, a species endemic to Banks Peninsula, had arisen from a dispersal event when the peninsula was still an island separated from the mainland. In his discussion of the distribution patterns of New Zealand stoneflies, McLellan (2006) attributed the much greater speciation in the South Island to the presence of refugia at various times during the Pleistocene ice age.

With respect to insect species more generally, Craw (1989) found that of 180 species with adequate data, 66 of those were restricted to the north of the country (north of Taupo), 57 to the north of the South Island and 59 to southern New Zealand (Otago and Southland). In the southern North Island and central South Island insects were mainly widespread species, rather than local endemics, a pattern shared with many snails and plants (Gibbs 2006). Suggested reasons for some of these patterns have been linked to the Pleistocene ice ages, which had a major effect in the central South Island leading to the annihilation of many species (Trewick & Wallis 2001; Gibbs 2006). Zones richer in biotic diversity at the two ends of the country and at the north of the South Island are perceived to have acted as refugia where speciation occurred and from which recolonisation of glaciated landscapes subsequently took place. The low diversity in the southern North Island may have been related to the presence of a wide sea strait (the Manawatu Strait) between the islands, and further to the north than the current Cook Strait, prior to glaciations. In the South Island movements of the alpine fault have also resulted in periods of mountain building that have acted as barriers to dispersal and huge volcanic eruptions in the North Island resulted in the extensive distribution of ash that resulted in the extirpation of many habitats and presumably the local extinction of species (Wyse et al. 2018).

The more extensive knowledge of the nationwide distributions of New Zealand mayflies I have attained confirm that a small number of species (32%) are found throughout the country, whereas others are restricted to the North or South islands, limited parts of them, or they tend to be widespread in the north and also present in the northwest of the South Island. Northland is widely perceived to have been an important faunal refuge during the Pleistocene ice ages and the richness of mayfly species there reflects its historical role (Smith & Collier 2001; Smith et al. 2006; Winterbourn et al. 2017).

Subsequently, species are thought to have recolonised streams and rivers further south by dispersal from these northern refuges. The extensive distribution patterns now shown by some mayfly species could suggest that they are strong dispersers, despite mayflies not having strong powers of flight and having winged stages with very short lives.

Towns and Peters (1980) speculated that the perceived low level of speciation shown by New Zealand mayflies might be attributed to a long isolation of an ancient specialised fauna, which has since undergone a low level of radiation and/or a high level of extinction. We now know that a much higher level of speciation has taken place in the New Zealand mayfly fauna, especially in the leptophlebiid genera *Deleatidium* and *Zephlebia*, and to a lesser extent *Nesameletus* (Nesameletidae). My molecular barcoding of species (Chapters 5 and 6; Appendix 2) has also demonstrated considerable variability in some widely distributed species (e.g. 4.7–5.6% in three *Nesameletus* species), suggesting the presence of possible cryptic species. Such findings support the notion that these widespread species are not strong dispersers, but show regional divergence not evident on distribution maps with the current level of knowledge.

A particularly strong finding of my study is that the two most diverse and common genera of Leptophlebiidae have very different distributions within the country. Thus, to generalise, *Zephlebia* is principally a North Island genus, whereas *Deleatidium* is found predominantly in the South Island. The nymphs of *Zephlebia* species also tend to occur in slow flowing parts of forest streams, whereas nymphs of many *Deleatidium* species are more characteristic of moderate to fast flowing waters, including unstable, physically disturbed streams and rivers found abundantly in much of the South Island (Towns & Peters 1996). Many of the species of *Deleatidium* now known in the South Island may have evolved in isolated river systems during periods of mountain building and glaciations, a situation that would parallel that found in the stonefly genus *Zelandobius* (McLellan 2006), and in some galaxiid fishes (McDowall 1990). Specialised cold water species of *Deleatidium* are also found in alpine and subalpine zones of the Southern Alps, an evolutionary development quite possibly unique in New Zealand mayflies. Three of these species belong to the subgenus *Penniketellum* and are potentially at risk from climate warming as their habitats recede. Although species of *Zephlebia* are primarily found in the North Island, three species also inhabit parts of the South Island. Interestingly, although *Z. spectabilis* occurs in many parts of the South Island, it is not found in the mountains but has an essentially coastal distribution (see map figure 1 at the end of the chapter) suggesting its distribution is limited by temperature and/or velocity. Two other *Zephlebia* species are found in the northwest of the South Island, one being an undescribed species that may have evolved in that region. The other, *Z. versicolor* has a New Zealand-wide distribution resembling that of *Siphlaenigma janae* (see Chapter 5) and may have been present at the top of the South Island prior to the opening of Cook Strait. Thus, Fleming (1962) showed how hypothetical river drainages from the north (cf. Whanganui, Rangitikei and Manawatu) during the

Pleistocene may have been in close proximity, or even connected to, drainages in the south (Tasman and Marlborough Regions). Interestingly, my preliminary molecular data (not presented) of pairwise genetic distances for *Z. versicolor* indicate an mean intraspecific distance of 5.9% between the North and South Island populations (5.5–6.5%; 12 species pairs from eight locations) suggesting the isolation of these populations, and possibly speciation in play.

Distribution Table 1 Species distribution grouped by the ecoregions of Harding and Winterbourn (1997). See Methods for aquatic ecoregion codes. Red text indicates locations of specimens collected during the nationwide survey (this research). Black text indicates locations recorded by other collections. An underlined code indicates a suspicious record that requires verification. Note: AU (Auckland Islands) was not a Harding and Winterbourn (1997) ecoregion but is included here for completeness.

Species recorded	Locations	North Island													South Island (including Stewart and Auckland Islands)												
		ND	CL	HP	BP	WO	TO	EH	EL	VP	TK	CM	MN	WA	NN	NP	NE	MP	WD	SA	HC	EC	PE	CO	SL	SE	AU
Ameletopsidae																											
Ameletopsis perscitus (Eaton, 1899)	57	ND	CL	HP	BP	WO	TO	EH	EL	VP	TK	CM	MN	WA	NN	NP	NE	MP	WD	SA	HC	EC	PE		SL	SE	
Coloburiscidae																											
Coloburiscus humeralis (Walker, 1853)	71	ND	CL	HP	BP	WO	TO	EH	EL	VP	TK	CM	MN	WA	NN	NP	NE	MP	WD	SA	HC	EC	PE	CO	SL	SE	
Ichthybotidae																											
Ichthybotus bicolor Tillyard, 1923	10															NP	NE	MP	WD		HC	EC	PE		SL		
Ichthybotus hudsoni (McLachlan, 1894)	26	ND	CL	HP	BP	WO	TO	EH		VP	TK	CM	MN	WA													
Leptophlebiidae																											
Acanthophlebia cruentata (Hudson, 1904)	33	ND	CL	HP	BP	WO	TO	EH	EL	VP	TK	CM	MN	WA													
Arachnocolus phillipsi Towns & Peters, 1979	19	ND	CL	HP	BP	WO	TO					CM	MN														
Atalophlebioides cromwelli (Phillips, 1930)	18	ND	CL		BP	WO		EH	EL				MN		NN	NP	NE	MP	WD	SA	HC	EC	PE	CO		SE	
Aupouriella pohei Winterbourn, 2009	0	ND																									
Austroclima jollyae Towns & Peters, 1979	25	ND	CL			WO	TO				TK	CM			NN	NP	NE	MP	WD	SA	HC	EC	PE	CO	SL	SE	
Austroclima sepia (Phillips, 1930)	38	ND	CL	HP	BP	WO	TO	EH	EL	VP	TK	CM	MN	WA	NN	NP	NE	MP	WD	SA	HC		PE			SE	
Austronella planulata (Towns, 1983)	13	ND			BP	WO	TO		EL			CM	MN	WA													
Cryophlebia aucklandensis (Peters, 1971)	0																										AU
Deleatidium (*D.*) *acerbum* Hitchings & Hitchings, 2016	1															NP			WD								
Deleatidium (*D.*) *angustum* Towns & Peters, 1996	22	ND	CL	HP		WO	TO			VP		CM	MN	WA	NN		NE		WD	SA	HC	EC	PE				
Deleatidium (*D.*) *atricolor* Hitchings, 2009	9															NP				SA	HC	EC		CO			
Deleatidium (*D.*) *autumnale* Phillips, 1930	22	ND				WO	TO	EH	EL	VP	TK	CM	MN	WA	NN	NP	NE	MP	WD	SA	HC	EC	PE	CO	SL	SE	
Deleatidium (*D.*) *branchiola* Hitchings, 2009	0																				HC						
Deleatidium (*D.*) *cerinum* Phillips, 1930	14	ND	CL		BP	WO	TO		EL			CM		WA	NN	NP	NE		WD	SA	HC	EC	PE	CO	SL	SE	
Deleatidium (*D.*) *fumosum* Phillips, 1930	49	ND	CL	HP	BP	WO	TO	EH	EL		TK	CM	MN	WA	NN	NP	NE	MP	WD	SA	HC	EC	PE	CO	SL	SE	
Deleatidium (*D.*) *kawatiri* Hitchings, 2016	0														NN				WD								
Deleatidium (*D.*) *kiwa* Hitchings, 2010	5																			SA						SE	
Deleatidium (*D.*) *lillii* Eaton, 1899	29	ND	CL		BP	WO	TO	EH	EL	VP	TK	CM		WA	NN	NP	NE	MP	WD	SA	HC	EC	PE	CO	SL	SE	
Deleatidium (*D.*) "lyellensis"	1																		WD								
Deleatidium (*D.*) *magnum* Towns & Peters, 1996	1						TO			VP	TK					NP					HC						
Deleatidium (*D.*) *myzobranchia* Phillips, 1930	50	ND	CL	HP		WO	TO		EL	VP	TK	CM		WA	NN	NP	NE	MP	WD	SA	HC	EC	PE	CO	SL	SE	
Deleatidium (*D.*) "rakiura"	3																									SE	
Deleatidium (*D.*) *townsi* Hitchings, 2009	16														NN	NP	NE	MP	WD	SA	HC						
Deleatidium (*D.*) *vernale* Phillips, 1930	25	ND	CL				TO		EL	VP		CM		WA	NN	NP	NE		WD	SA	HC	EC	PE	CO	SL	SE	

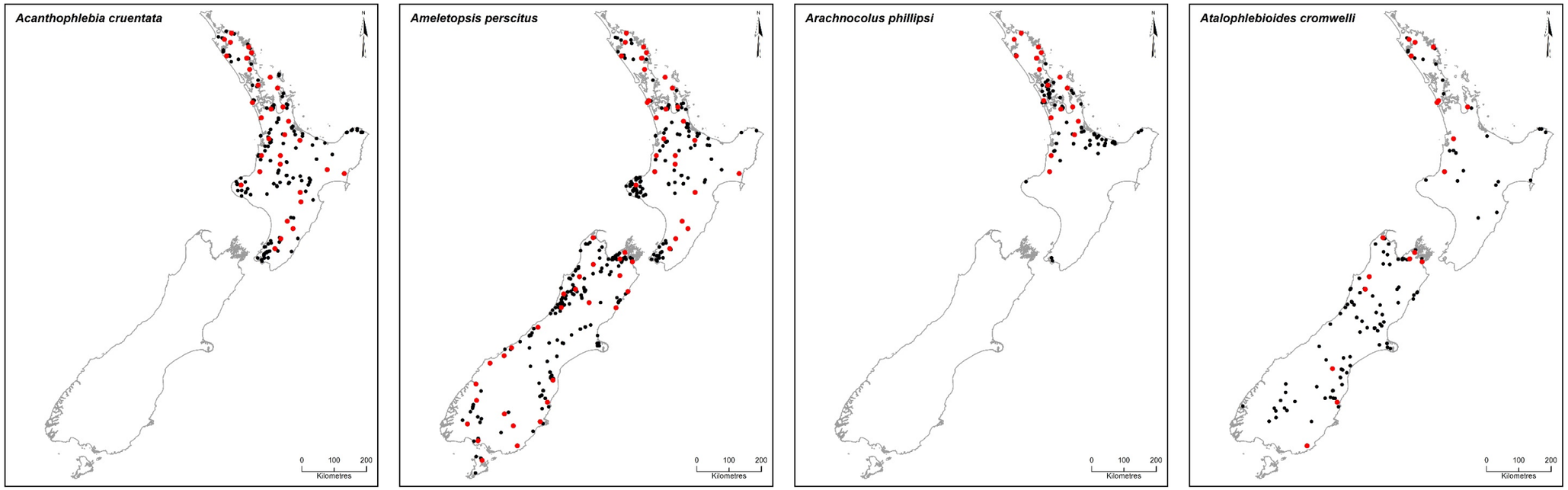
Species recorded	Locations	North Island													South Island (including Stewart and Auckland Islands)												
		ND	CL	HP	BP	WO	TO	EH	EL	VP	TK	CM	MN	WA	NN	NP	NE	MP	WD	SA	HC	EC	PE	CO	SL	SE	AU
Deleatidium (*D.*) *wardorum* Hitchings, 2010	13														NN	NP	NE	MP	WD	SA	HC	EC		CO			
Deleatidium (*P.*) *cornutum* Towns & Peters, 1996	1														NN					SA	HC						
Deleatidium (*P.*) *insolitum* (Towns & Peters, 1979)	0																			SA	HC						
Deleatidium (*P.*) *patricki* Hitchings, 2008	1															NP				SA	HC						
Isothraulus abditus Towns & Peters, 1979	14	ND	CL			WO	TO																				
Mauiulus aquilus Towns & Peters, 1996	1	ND	CL			WO	TO		EL																		
Mauiulus luma Towns & Peters, 1979	20	ND	CL	HP		WO	TO	EH	EL	VP		CM	MN		NN			MP	WD								
Neozephlebia scita (Walker, 1853)	56	ND	CL	HP	BP	WO	TO	EH	EL	VP	TK	CM	MN	WA	NN	NP	NE	MP	WD	SA	HC	EC	PE		SL	SE	
Tepakia caligata Towns & Peters, 1996	4	ND	CL	HP	BP	WO	TO						MN	WA													
Zephlebia borealis (Phillips, 1930)	28	ND	CL	HP	BP	WO	TO				TK	CM	MN														
Zephlebia dentata (Eaton, 1871)	33	ND	CL	HP	BP	WO	TO	EH	EL	VP	TK	CM	MN	WA													
Zephlebia inconspicua Towns, 1983	15	ND	CL	HP		WO																					
Zephlebia nebulosa Towns & Peters, 1996	7	ND	CL			WO	TO	EH							NN		NE										
Zephlebia pirongia Towns & Peters, 1996	1			HP		WO																					
Zephlebia aff. *pirongia* sp. 1	1	ND																									
Zephlebia spectabilis Towns, 1983	41	ND	CL			WO	TO		EL	VP	TK	CM		WA	NN	NP	NE		WD		HC		PE		SL	SE	
Zephlebia tuberculata Towns & Peters, 1996	13	ND	CL		BP	WO	TO	EH					MN														
Zephlebia versicolor (Eaton, 1899)	37	ND	CL		BP	WO	TO	EH	EL	VP	TK	CM	MN	WA	NN	NP	NE	MP	WD								
Zephlebia sp. 1	10														NN	NP	NE	MP	WD								
Nesameletidae																											
Nesameletus austrinus Hitchings & Staniczek, 2003	11														NN	NP			WD	SA	HC						
Nesameletus flavitinctus (Tillyard, 1923)	21	ND	CL	HP	BP	WO	TO	EH		VP	TK	CM			NN	NP	NE	MP	WD	SA	HC				SL		
Nesameletus murihiku Hitchings & Staniczek, 2003	1																		WD	SA	HC					SE	
Nesameletus ornatus (Eaton, 1883)	46	ND	CL	HP	BP	WO	TO	EH	EL	VP	TK	CM	MN	WA	NN	NP	NE	MP	WD	SA	HC	EC	PE	CO	SL	SE	
Nesameletus vulcanus Hitchings & Staniczek, 2003	0																						PE				
Nesameletus "fiordlandi"	3																		WD	SA							
Oniscigastridae																											
Oniscigaster distans Eaton, 1899	21						TO			VP	TK	CM			NN	NP	NE	MP	WD	SA	HC	EC			SL		
Oniscigaster wakefieldi McLachlan, 1873	11	ND				WO	TO		EL			CM		WA		NP	NE		WD		HC				SL		
Rallidentidae																											
Rallidens mcfarlanei Penniket, 1966	13	ND	CL		BP	WO	TO	EH			TK	CM		WA					WD								
Rallidens platydontis Staniczek & Hitchings, 2014	2															NP	NE	MP	WD		HC	EC			SL		
Siphlaenigmatidae																											
Siphlaenigma janae Penniket, 1962	10	ND	CL		BP	WO	TO	EH		VP									WD								

Distribution Table 2 Mayfly species distribution grouped by the New Zealand biogeographical zones of Crosby et al. (1998). See Methods for biogeographical zone codes. Red text indicates locations of specimens collected during the nationwide survey of this research. Black text indicates locations recorded by other collections. An underlined code indicates a suspicious record that requires verification. **Note:** No survey locations were positioned in the HB or WI biogeographical zones.

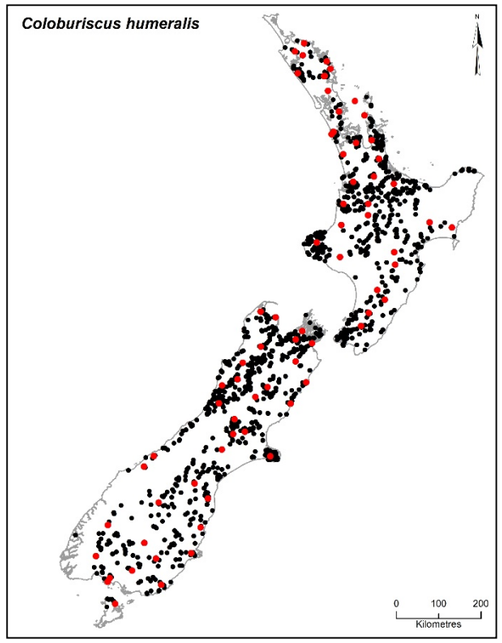
Species recorded	**North Island**													**South Island (including Stewart and Auckland Islands)**															
	ND	**AK**	**CL**	**WO**	**BP**	**TK**	**TO**	**GB**	**HB**	**RI**	**WI**	**WN**	**WA**	**SD**	**NN**	**BR**	**WD**	**MB**	**KA**	**NC**	**MC**	**SC**	**MK**	**OL**	**CO**	**DN**	**SL**	**FD**	**SI**	**AU**
Ameletopsidae																														
Ameletopsis perscitus (Eaton, 1899)	ND	AK	CL	WO	BP	TK	TO	GB		RI		WN	WA	SD	NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO	DN	SL	FD	SI	
Coloburiscidae																														
Coloburiscus humeralis (Walker, 1853)	ND	AK	CL	WO	BP	TK	TO	GB	HB	RI	WI	WN	WA	SD	NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO	DN	SL	FD	SI	
Ichthybotidae																														
Ichthybotus bicolor Tillyard, 1923												WN		SD	NN	BR	WD	MB	KA	NC	MC	SC		OL	CO	DN				
Ichthybotus hudsoni (McLachlan, 1894)	ND	AK	CL	WO	BP	TK	TO	GB	HB	RI	WI	WN	WA																	
Leptophlebiidae																														
Acanthophlebia cruentata (Hudson, 1904)	ND	AK	CL	WO	BP	TK	TO	GB	HB	RI		WN	WA																	
Arachnocolus phillipsi Towns & Peters, 1979	ND	AK	CL	WO	BP	TK						WN																		
Atalophlebioides cromwelli (Phillips, 1930)	ND	AK	CL	WO	BP	TK	TO	GB	HB	RI		WN		SD	NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO	DN	SL	FD		
Aupouriella pohei Winterbourn, 2009	ND																													
Austroclima jollyae Towns & Peters, 1979	ND	AK	CL	WO	BP	TK	TO					WN		SD	NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO	DN	SL	FD	SI	
Austroclima sepia (Phillips, 1930)	ND	AK	CL	WO	BP	TK	TO	GB	HB	RI	WI	WN	WA	SD	NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO	DN	SL	FD	SI	
Austronella planulata (Towns, 1983)	ND	AK		WO	BP	TK	TO	GB	HB	RI		WN	WA																	
Cryophlebia aucklandensis (Peters, 1971)																														AU
Deleatidium (*D.*) *acerbum* Hitchings & Hitchings, 2016															NN	BR	WD													
Deleatidium (*D.*) *angustum* Towns & Peters, 1996	ND	AK	CL	WO	BP	TK	TO	GB	HB	RI		WN	WA	SD	NN		WD		KA		MC	SC			CO		SL			
Deleatidium (*D.*) *atricolor* Hitchings, 2009																BR	WD	MB	KA	NC	MC	SC	MK	OL	CO		SL	FD		
Deleatidium (*D.*) *autumnale* Phillips, 1930	ND	AK		WO	BP	TK	TO	GB	HB	RI		WN	WA	SD	NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO	DN	SL	FD		
Deleatidium (*D.*) *branchiola* Hitchings, 2009																								OL	CO					
Deleatidium (*D.*) *cerinum* Phillips, 1930	ND	AK		WO	BP	TK	TO	GB		RI		WN	WA	SD	NN	BR	WD	MB		NC	MC	SC	MK	OL	CO	DN	SL	FD	SI	
Deleatidium (*D.*) *fumosum* Phillips, 1930	ND	AK	CL	WO	BP	TK	TO	GB		RI		WN	WA	SD	NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO	DN	SL	FD	SI	
Deleatidium (*D.*) *kawatiri* Hitchings, 2016															NN	BR														
Deleatidium (*D.*) *kiwa* Hitchings, 2010																											SL	FD	SI	
Deleatidium (*D.*) *lillii* Eaton, 1899	ND	AK	CL	WO	BP	TK	TO			RI		WN	WA	SD	NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO	DN	SL	FD	SI	
Deleatidium (*D.*) "lyellensis"															NN															
Deleatidium (*D.*) *magnum* Towns & Peters, 1996						TK	TO								NN					NC	MC	SC	MK		CO					
Deleatidium (*D.*) *myzobranchia* Phillips, 1930	ND	AK	CL	WO	BP	TK	TO			RI		WN	WA	SD	NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO	DN	SL	FD	SI	
Deleatidium (*D.*) "rakiura"																													SI	
Deleatidium (*D.*) *townsi* Hitchings, 2009														SD	NN	BR	WD	MB	KA	NC	MC	SC	MK							
Deleatidium (*D.*) *vernale* Phillips, 1930	ND		CL				TO	GB		RI		WN	WA	SD	NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO	DN	SL	FD		

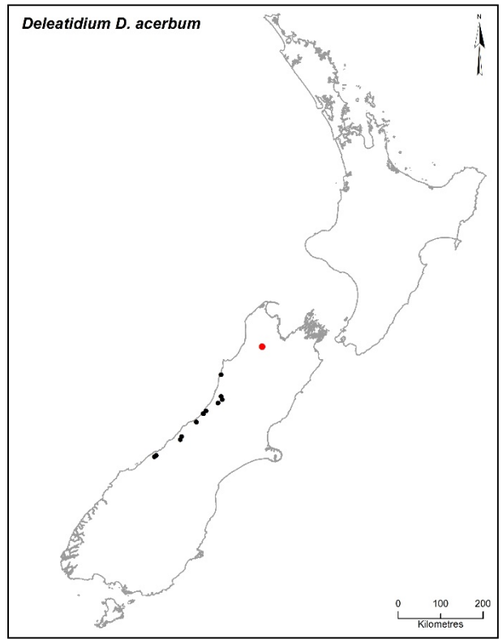
Species recorded	North Island													South Island (including Stewart and Auckland Islands)															
	ND	AK	CL	WO	BP	TK	TO	GB	HB	RI	WI	WN	WA	SD	NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO	DN	SL	FD	SI	AU
Deleatidium (*D.*) *wardorum* Hitchings, 2010														SD	NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO					
Deleatidium (*P.*) *cornutum* Towns & Peters, 1996																BR	WD	MB	KA	NC	MC	SC	MK	OL	CO	DN		FD		
Deleatidium (*P.*) *insolitum* (Towns & Peters, 1979)																				NC			MK							
Deleatidium (*P.*) *patricki* Hitchings, 2008															NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO			FD		
Isothraulus abditus Towns & Peters, 1979	ND	AK	CL	WO	BP	TK	TO			RI																				
Mauiulus aquilus Towns & Peters, 1996	ND		CL	WO			TO						WA																	
Mauiulus luma Towns & Peters, 1979	ND	AK		WO	BP	TK	TO	GB		RI		WN			NN	BR	WD	MB												
Neozephlebia scita (Walker, 1853)	ND	AK	CL	WO	BP	TK	TO	GB		RI		WN	WA	SD	NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO	DN	SL	FD	SI	
Tepakia caligata Towns & Peters, 1996	ND	AK	CL	WO	BP	TK	TO	GB				WN	WA																	
Zephlebia borealis (Phillips, 1930)	ND	AK	CL	WO	BP	TK	TO	GB		RI		WN																		
Zephlebia dentata (Eaton, 1871)	ND	AK	CL	WO	BP	TK	TO	GB		RI	WI	WN	WA																	
Zephlebia inconspicua Towns, 1983	ND	AK	CL	WO	BP		TO																							
Zephlebia nebulosa Towns & Peters, 1996	ND	AK	CL	WO	BP	TK	TO							SD		BR														
Zephlebia pirongia Towns & Peters, 1996				WO																										
Zephlebia aff. *pirongia* sp. 1	ND																													
Zephlebia spectabilis Towns, 1983	ND	AK	CL	WO	BP	TK	TO	GB		RI		WN	WA	SD	NN	BR	WD	MB	KA		MC	SC		OL	CO	DN	SL	FD	SI	
Zephlebia tuberculata Towns & Peters, 1996	ND	AK	CL	WO	BP	TK		GB		RI																				
Zephlebia versicolor (Eaton, 1899)	ND	AK	CL	WO	BP	TK	TO	GB		RI		WN	WA	SD	NN	BR		MB												
Zephlebia sp. 1														SD	NN	BR	WD	MB										FD		
Nesameletidae																														
Nesameletus austrinus Hitchings & Staniczek, 2003															NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO		SL	FD		
Nesameletus flavitinctus (Tillyard, 1923)	ND	AK	CL	WO	BP	TK	TO	GB	HB	RI		WN		SD	NN	BR	WD	MB	KA	NC	MC	SC		OL	CO	DN				
Nesameletus murihiku Hitchings & Staniczek, 2003																								OL	CO		SL	FD	SI	
Nesameletus ornatus (Eaton, 1883)	ND	AK	CL	WO	BP	TK	TO	GB	HB	RI		WN	WA	SD	NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO	DN	SL	FD		
Nesameletus vulcanus Hitchings & Staniczek, 2003																					MC									
Nesameletus “fiordlandi”																	WD											FD		
Oniscigastridae																														
Oniscigaster distans Eaton, 1899						TK	TO	GB		RI		WN		SD	NN	BR	WD	MB	KA	NC	MC	SC	MK	OL	CO	DN	SL	FD	SI	
Oniscigaster wakefieldi McLachlan, 1873	ND	AK		WO		TK	TO	GB				WN	WA	SD	NN	BR	WD	MB	KA	NC				OL	CO	DN	SL			
Rallidentidae																														
Rallidens mcfarlanei Penniket, 1966	ND	AK	CL	WO	BP	TK	TO			RI			WA			BR														
Rallidens platydontis Staniczek & Hitchings, 2014															NN			MB		NC		SC		OL		DN	SL			
Siphlaenigmatidae																														
Siphlaenigma janae Penniket, 1962	ND	AK	CL		BP	TK	TO	GB		RI						BR														

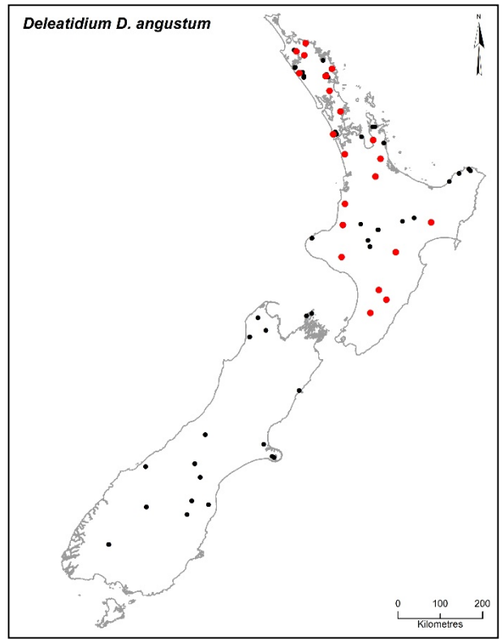
New Zealand mayfly distribution maps

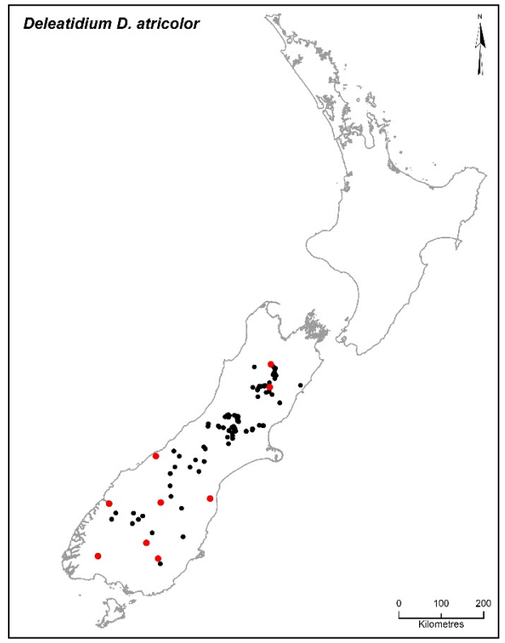


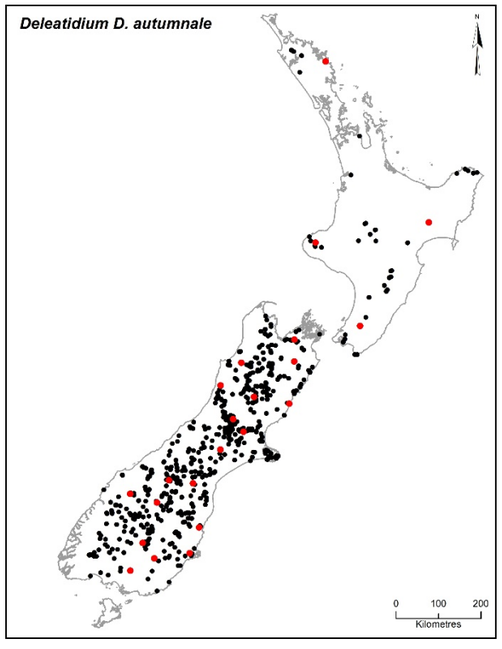
Map Figure Part 1 Distributions of 53 described New Zealand Ephemeroptera, and five tentative species. Species excluded are *Coloburiscus tonnoiri* and *Oniscigaster intermedius* for which no data is known. Species maps are arranged in alphabetical order except for *Cryophlebia aucklandensis* and *Nesameletus* "fiordlandi" which are presented last, due to the different map scales used. Distributions are generated from both my nationwide survey records (red dots) and the historical dataset (black dots). Maps are generated at a reasonable level of resolution to allow closer inspection with magnification tools.

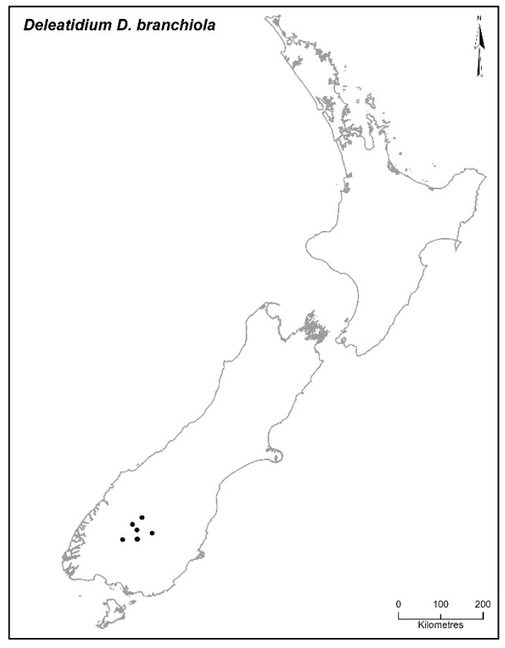


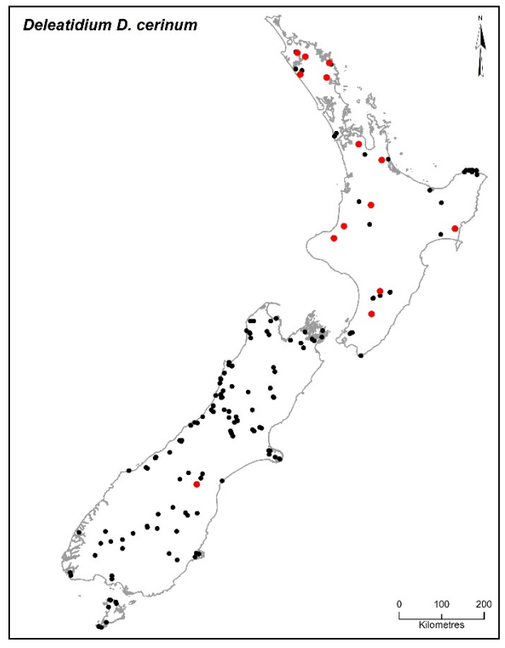


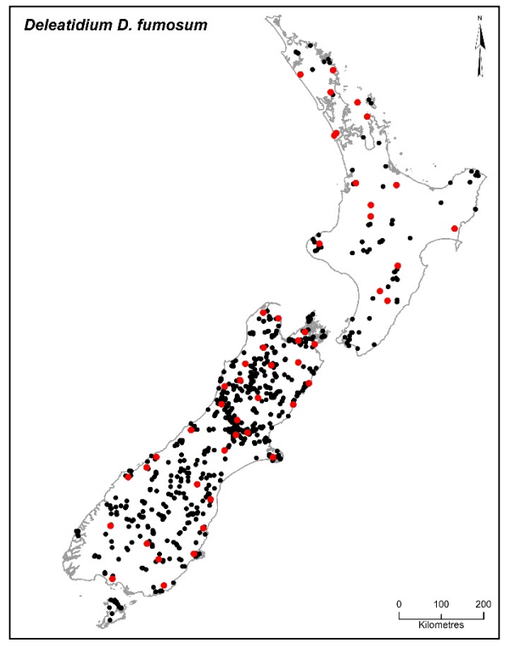




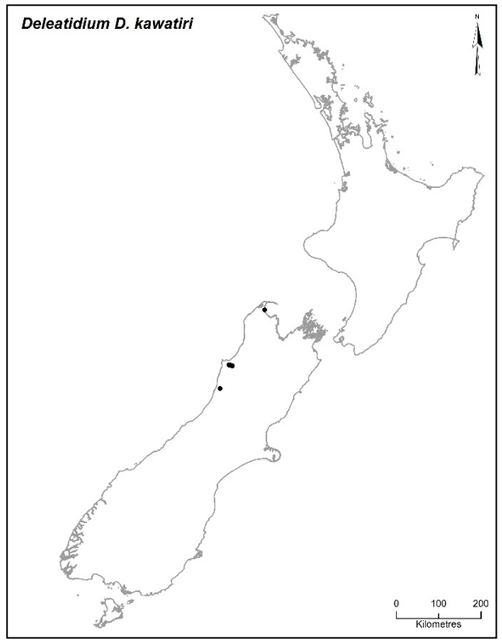


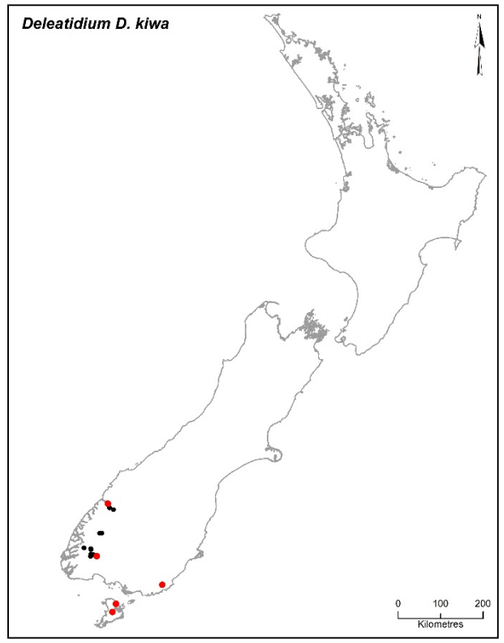


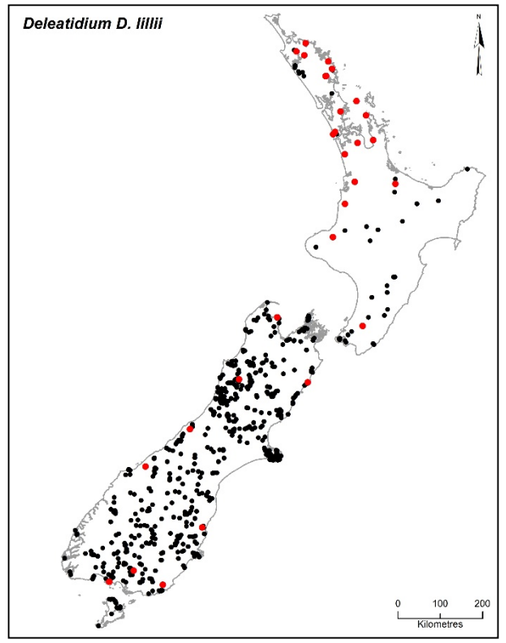


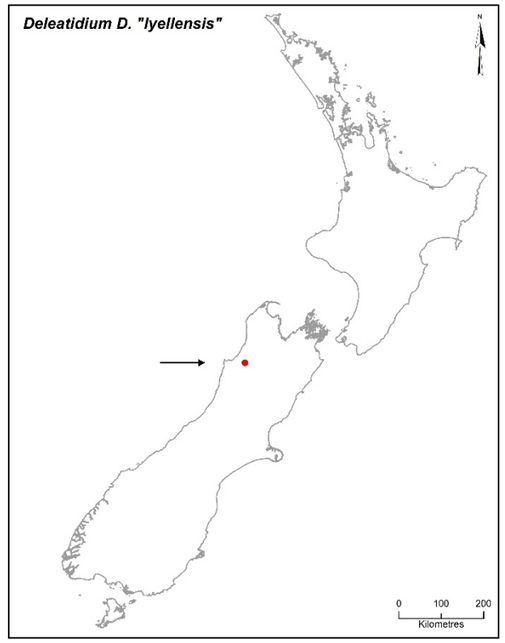


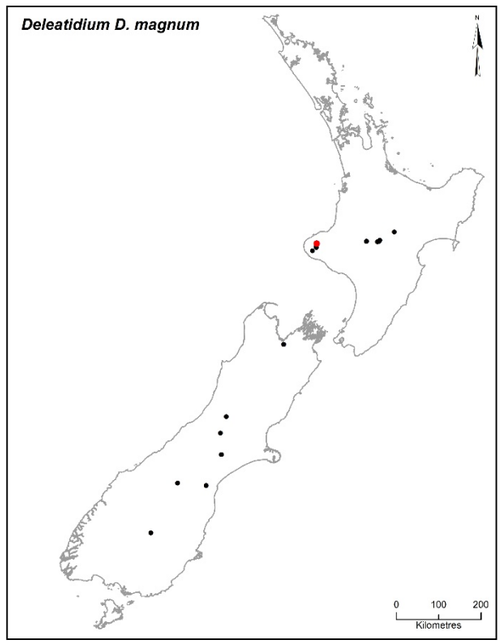
Map Figure Part 1 continued…

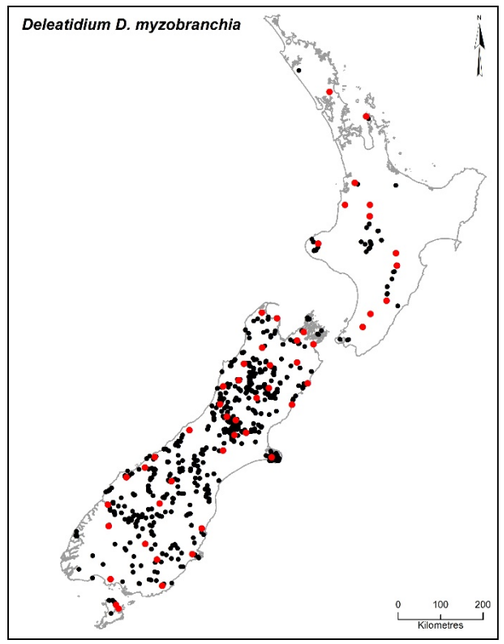


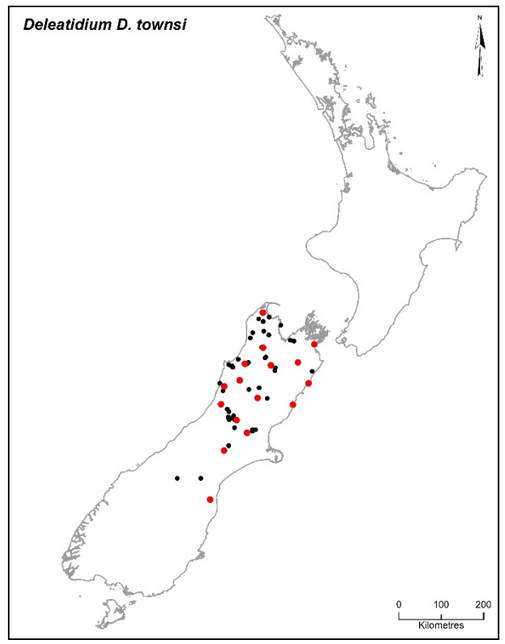












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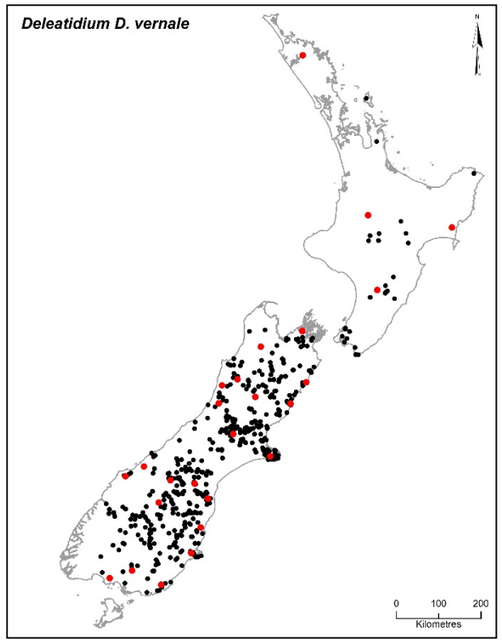

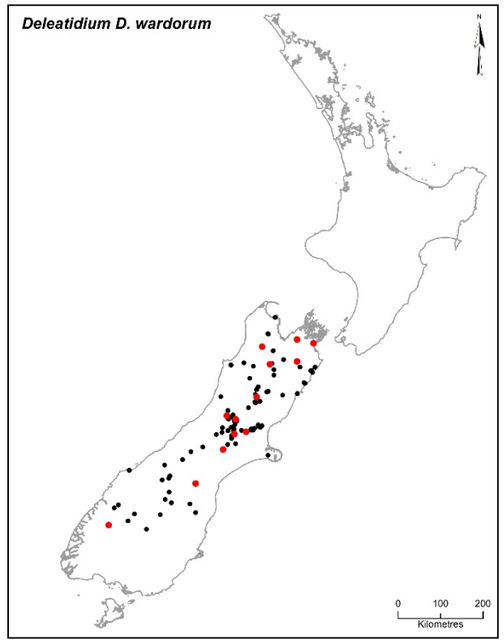

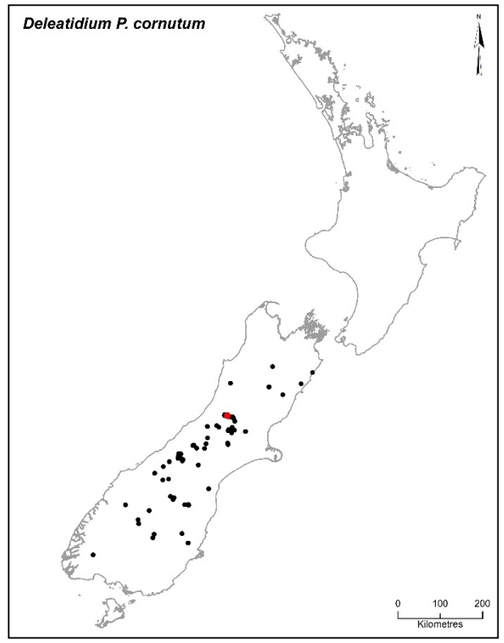

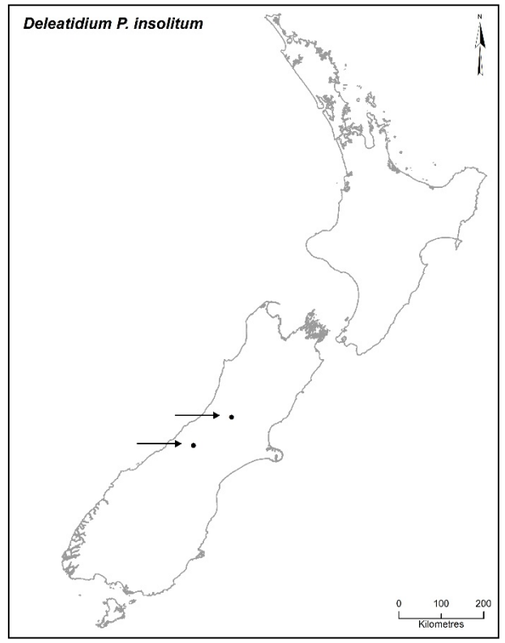

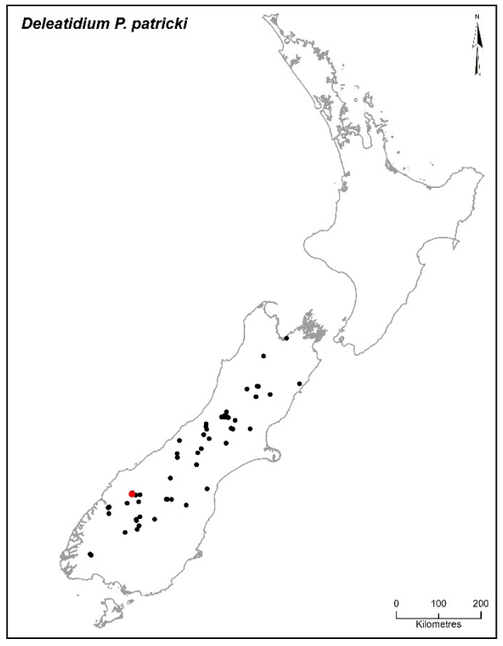

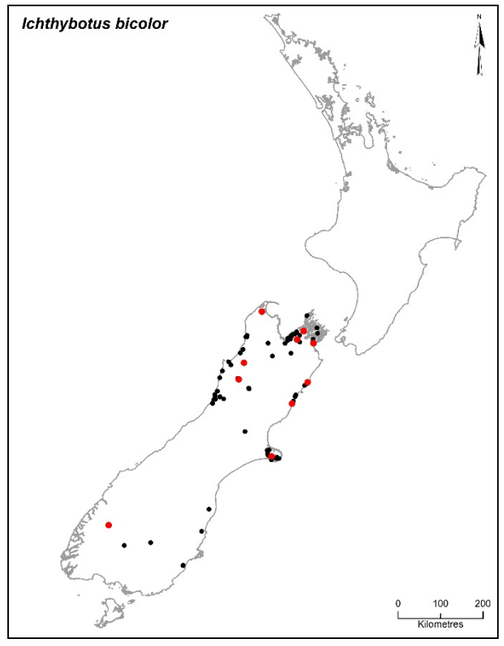

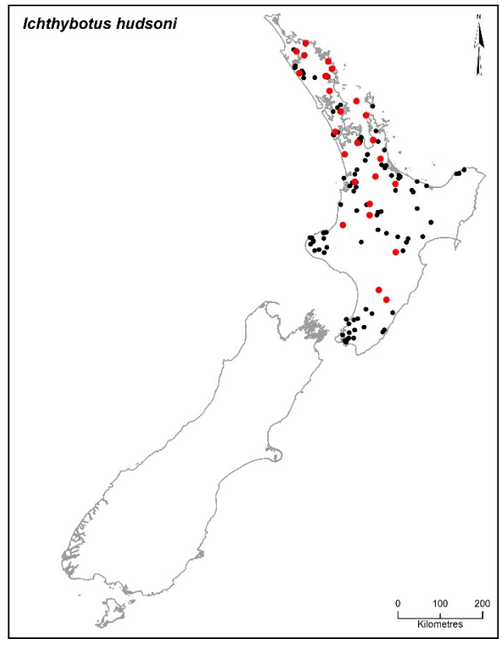

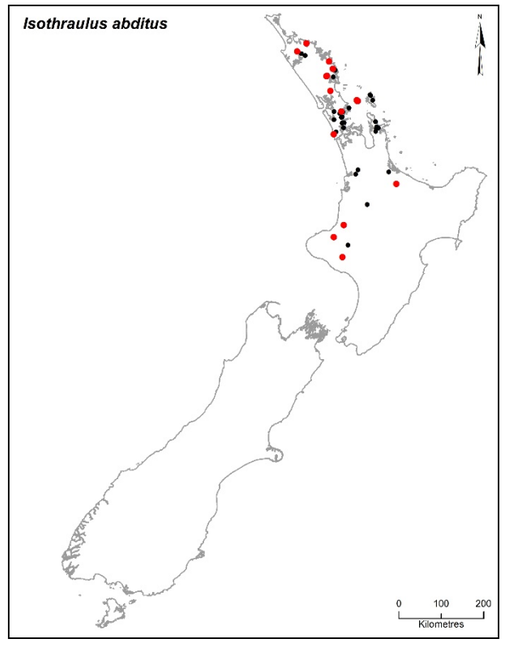

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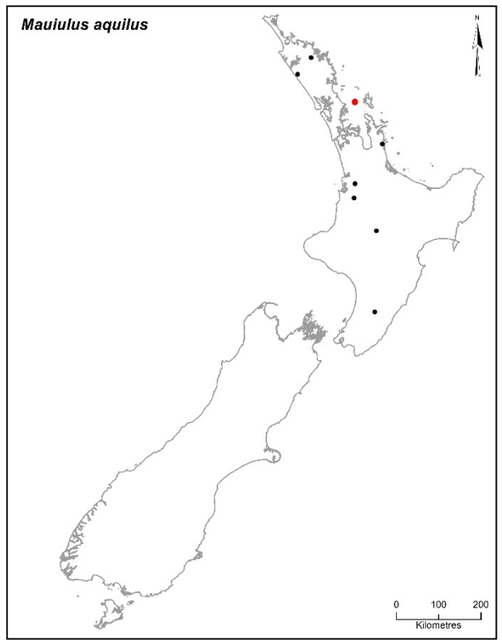

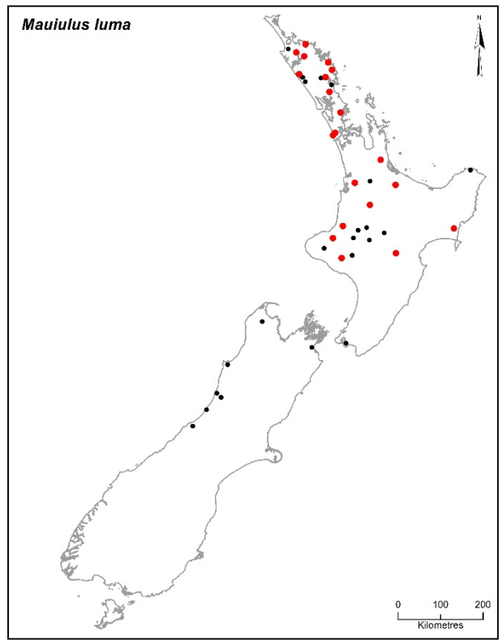

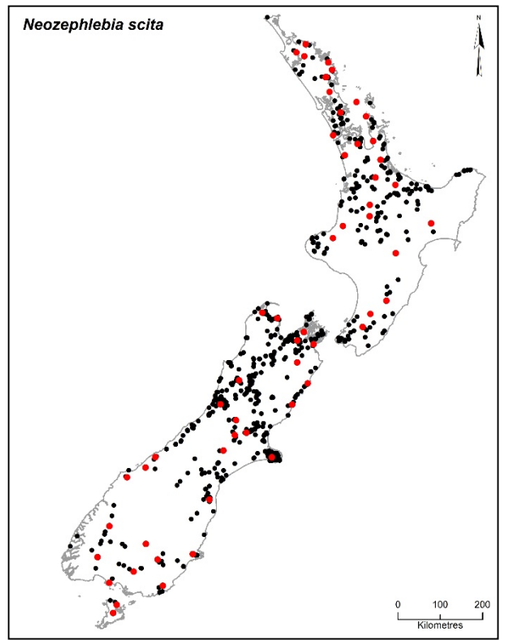

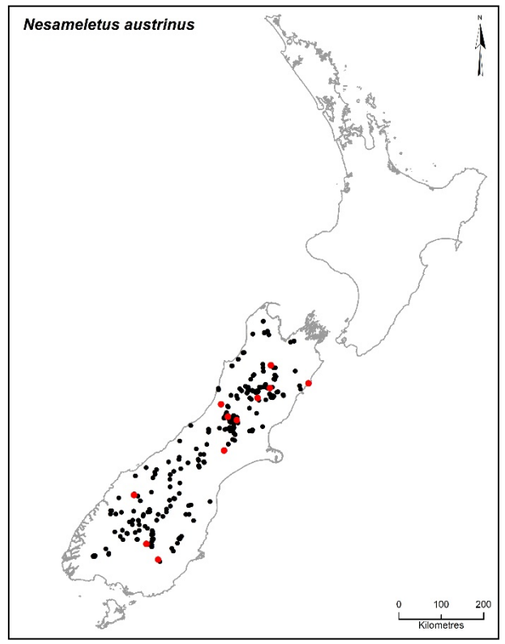

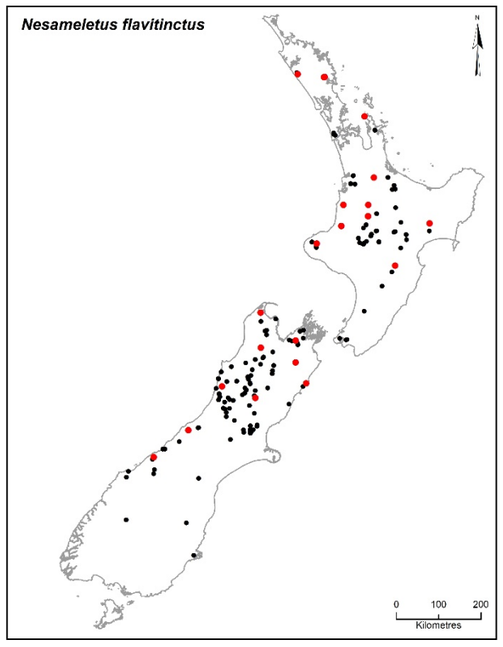

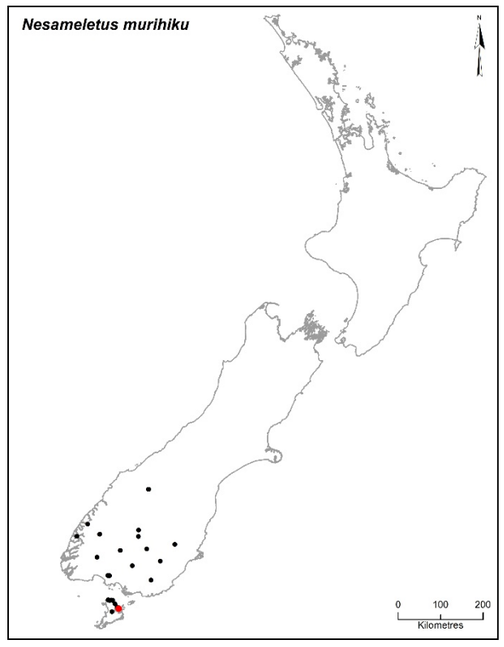

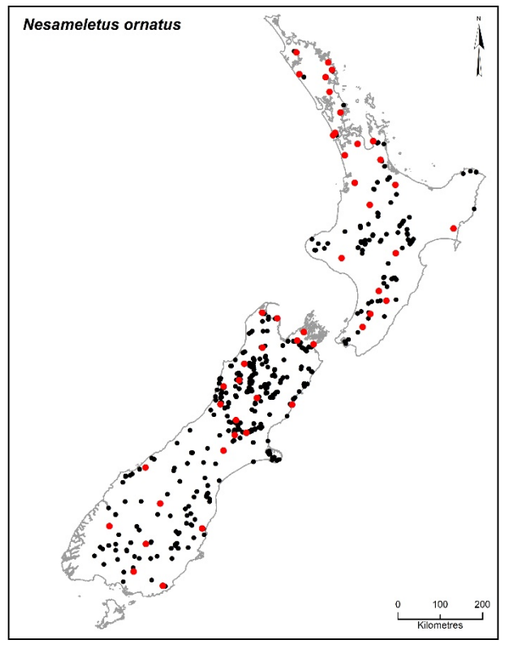

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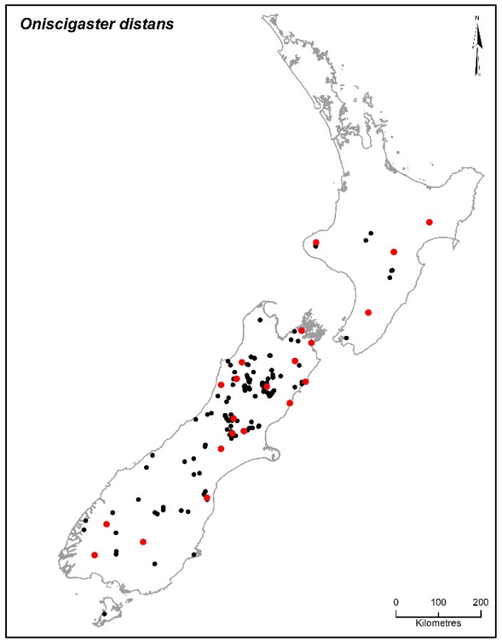

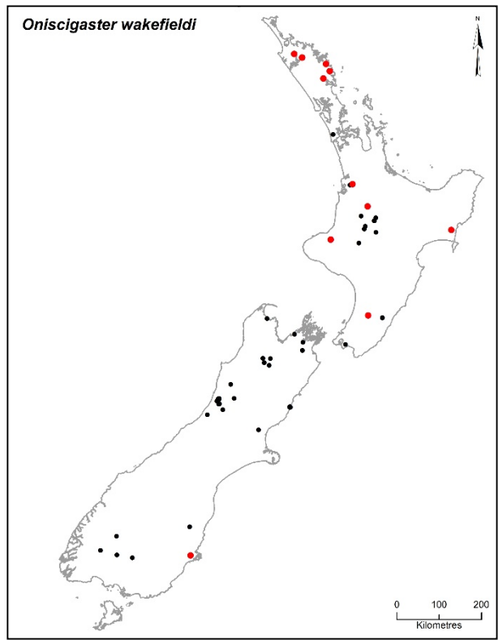

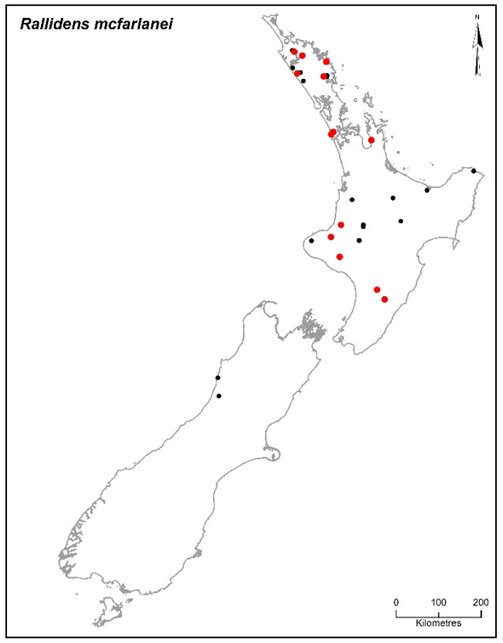

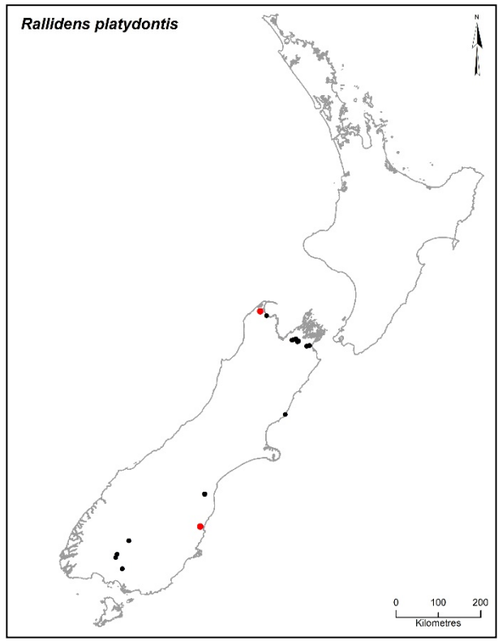

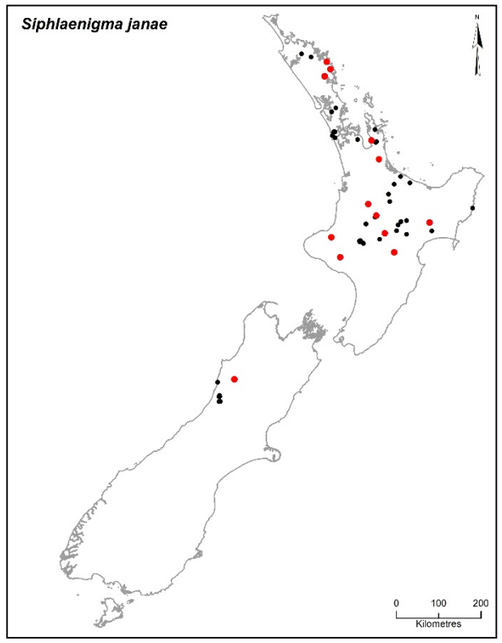

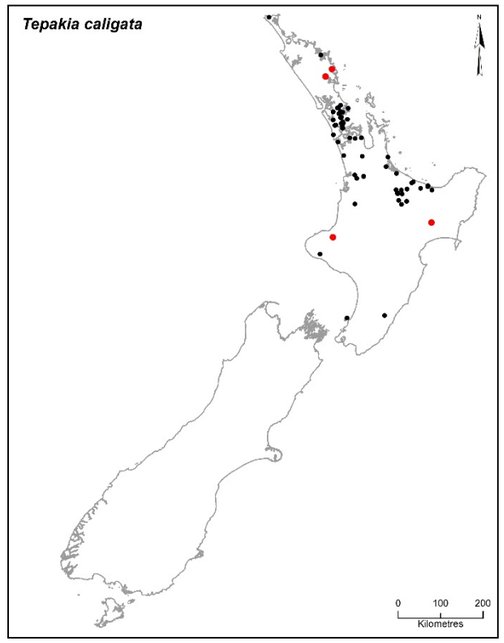

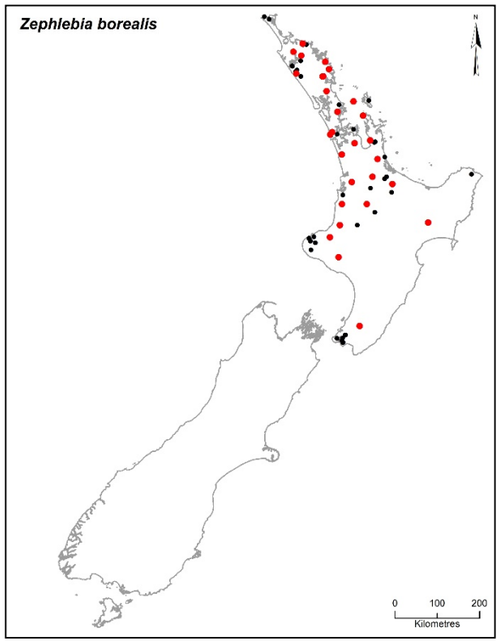

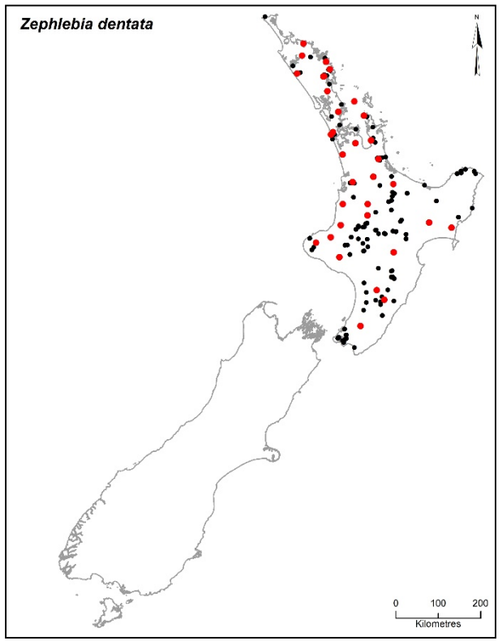

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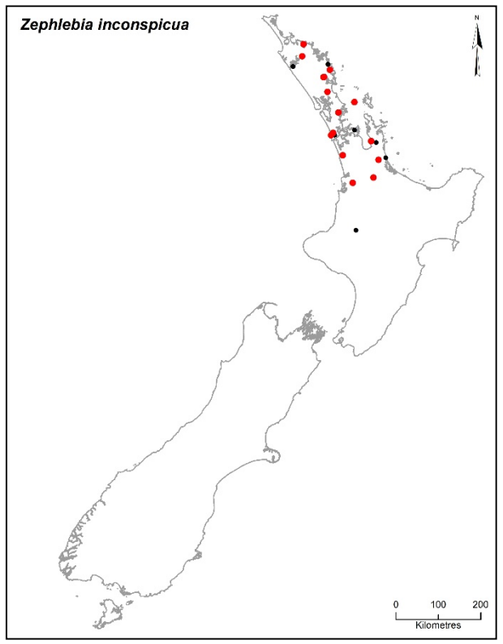
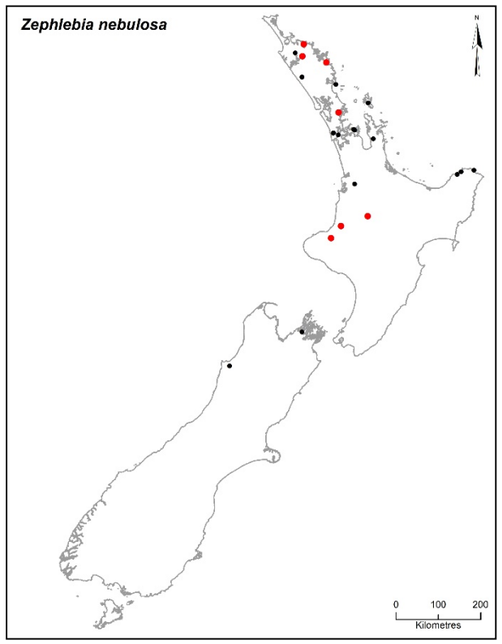
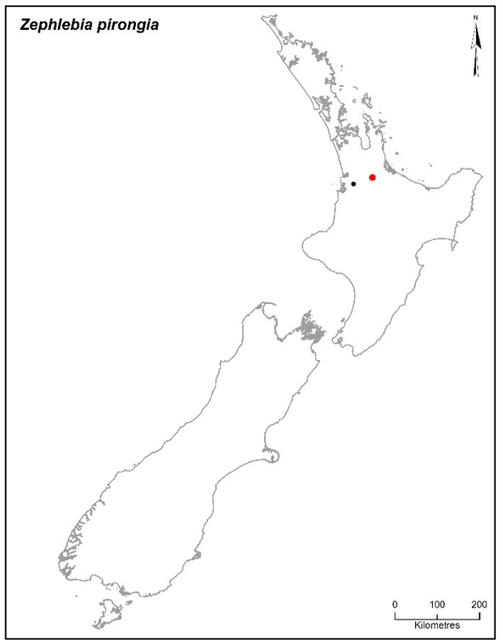
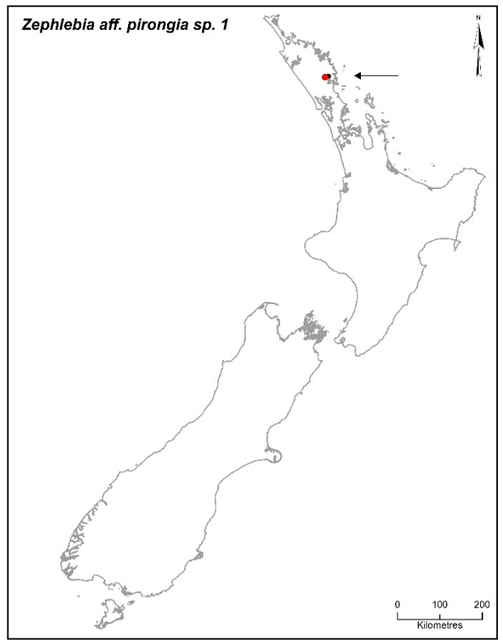
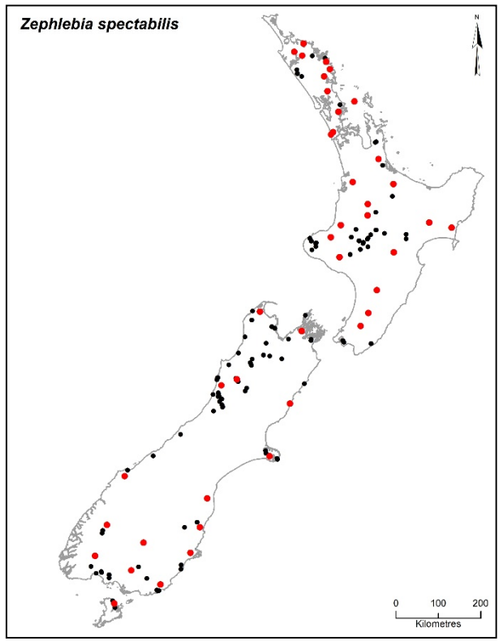
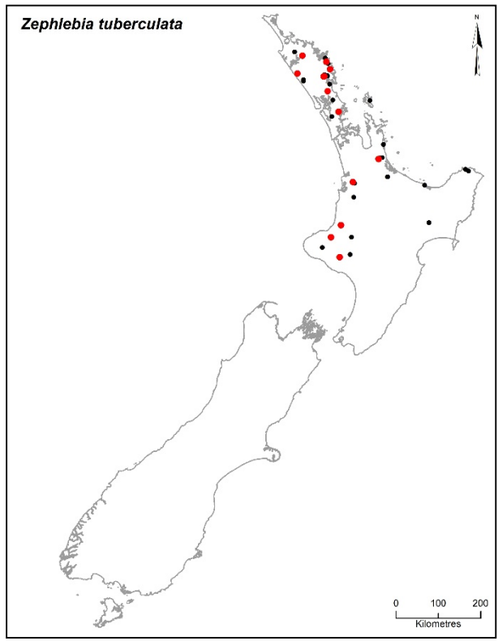


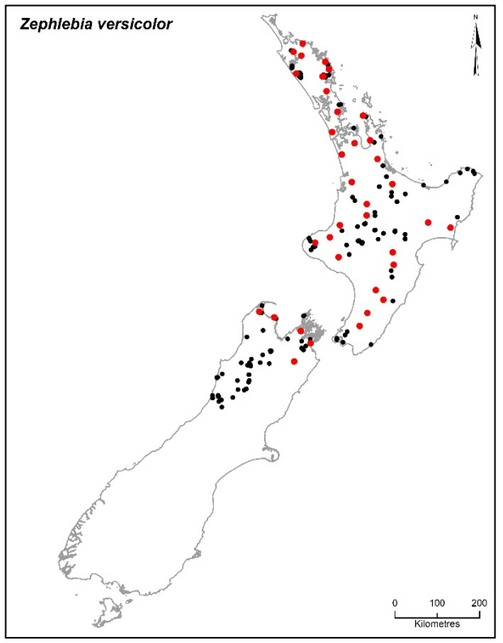


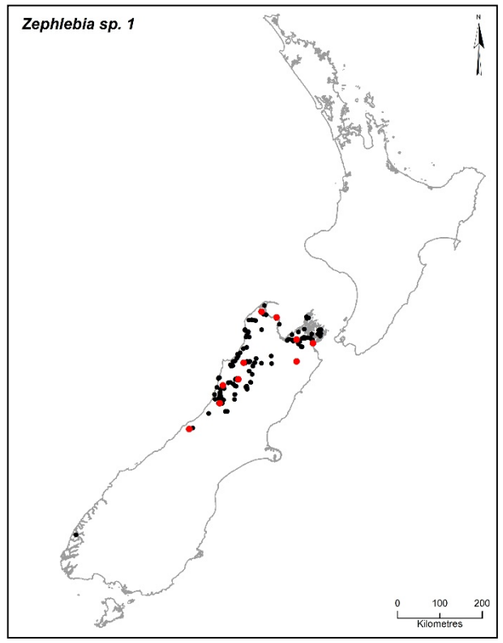




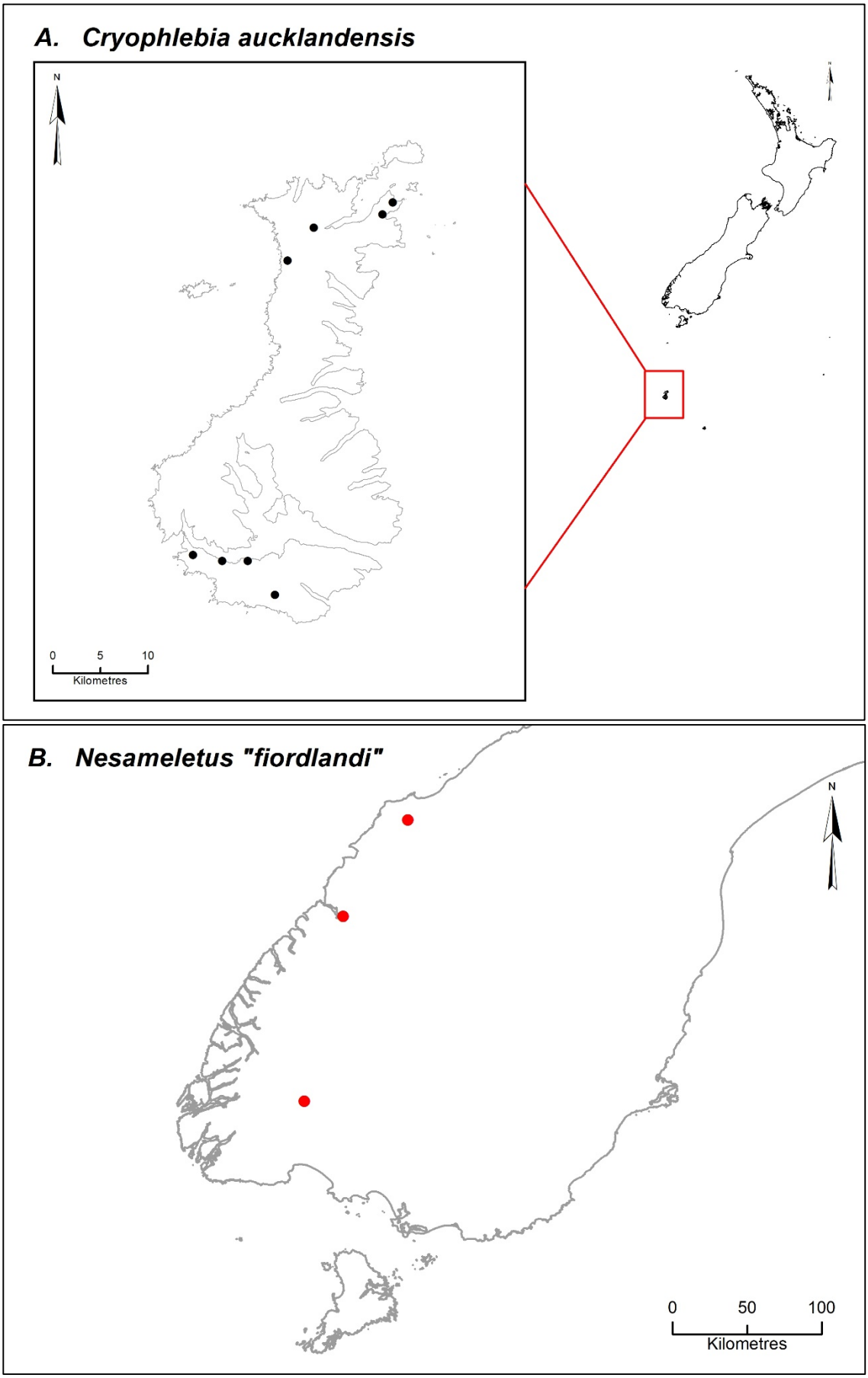








Map Figure Part 1 continued…



Map Figure Part 2 Distribution of **A.** *Cryophlebia aucklandensis*, Auckland Islands, southern Pacific Ocean and **B.** *Nesameletus* "fiordlandi" in the southern South Island.

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Chapter 5 – *Ecology and conservation of Siphlaenigma janae*

"If all mankind were to disappear, the world would regenerate back to the rich state of equilibrium that existed ten thousand years ago. If insects were to vanish, the environment would collapse into chaos."

Prof. Edward O. Wilson, 1992.

Subimago female of *Siphlaenigma janae* (Ephemeroptera). Photo credit: Olivier Ball / Stephen Pohe Collection.

Chapter 5 preface

This chapter describes a study of the poorly known species *Siphlaenigma janae* and has been published as Stephen R. Pohe, Michael J. Winterbourn, Sharyn J. Goldstien and Jon S. Harding. 2018. Distribution, body size, genetic structure and conservation of *Siphlaenigma janae* (Insecta: Ephemeroptera). New Zealand Journal of Zoology 45: 154–170. https://doi.org/10.1080/03014223.2017.1405823. The journal's style and conventions have been retained but the format of tables and figures have been adjusted to fit the broader thesis layout. I undertook the data collection and analyses, laboratory work and wrote the draft manuscript. My co-author Prof. Harding provided advice on study design, helped with the collection of specimens in the field, and commented on the final draft of the manuscript. Dr Goldstein oversaw genetic analyses in the laboratory, provided assistance with the genetic analyses and interpretation, and commented on drafts of the manuscript. Prof. Winterbourn provided advice on study design, assistance with genetic interpretations and manuscript writing, and commented on drafts of the manuscript.

Abstract

Distribution, body size and genetic structure of the poorly known New Zealand mayfly *Siphlaenigma janae* were investigated to improve understanding of its conservation status. It has now been recorded from 42 locations, 22 of which are reported for the first time. The distribution of *S. janae* extends from Northland to the central North Island, and also into the northwestern South Island. Population structure consisted of three distinct haplotype networks; two in the North Island and one in the South Island. Maximum uncorrected genetic distance was 6.1% but no strong evidence for the presence of sibling species was found. The specialist nymphs predominantly live in low-gradient first- and second-order forested streams, a habitat that is increasingly being threatened by land-use modification. We recommend *S. janae* remain classified as Nationally Vulnerable due to its unique taxonomic position, regionally distinct genetic structure and fragmented population that is likely experiencing ongoing decline.

Introduction

The endemic New Zealand mayfly *Siphlaenigma janae* Penniket, 1962 is the only described species of the family Siphlaenigmatidae (Penniket 1962). The type series of specimens were collected from a small stream, informally known as 'Baxter's Creek', which flowed into the Arnold River on the West Coast of the South Island. It was the only known location of *S. janae* at the time. The taxonomic status and phylogenetic relationships of Siphlaenigmatidae have been the subjects of some debate. In establishing the family, Penniket (1962) showed that it possessed numerous morphological characters, including aspects of wing venation, external genitalia, eyes and legs that were intermediate between those of Baetidae and Siphlonuridae, as defined at the time. However, Riek (1973) considered it to be a subfamily

of Baetidae (i.e. Siphlaenigmatinae) and both McCafferty and Edmunds (1979) and Landa and Soldán (1985) supported this change. Subsequently, McCafferty (1991) accorded the taxon family status once again and more recent workers have accepted that designation. The phylogenetic position of Siphlaenigmatidae within Ephemeroptera is not clear. A detailed morphological study by Staniczek (1997) indicated that *S. janae* has retained many plesiomorphic baetoid characters and supported the contention that Baetidae and Siphlaenigmatidae were sister groups. The results of a study based on 101 morphological characters by Ogden et al. (2009) also placed the two families together, but their phylogeny based on DNA sequences from five genes grouped the Siphlaenigmatidae with several other families, including two with New Zealand representatives (Coloburiscidae and Ameletopsidae), and not with Baetidae (Ogden et al. 2009).

Knowledge of the distribution of *S. janae* was expanded by Towns (1978) who provided the first records of its occurrence in the North Island. He found nymphs in slow-flowing reaches of forest streams in the Waitakere Ranges west of Auckland and also listed the localities of several specimens present in museum collections. A second species reputed to be from Australia was described from nymphs by Lugo-Ortiz and McCafferty (1998), but was subsequently synonymised with *S. janae*, when the material was found to have actually come from New Zealand (McCafferty 1999). More recently, Hitchings (2001) and Hitchings et al. (2015) published maps showing the collection sites of material held in the Canterbury Museum. The maps indicate a fragmented distribution and the species was listed as Nationally Vulnerable by Grainger et al. (2014). Except for a description of the egg by Koss and Edmunds (1974) and observations made by Towns (1978) on the general habitat of nymphs, including their propensity to be associated with algae or terrestrial vegetation trailing in the water, nothing is known about the life history and ecology of *S. janae*.

The specific aims of the present study were to reassess the conservation status of *S. janae* based on (1) an update of the known distribution and habitat; (2) an investigation of body size variation within the species; and (3) a preliminary examination of the genetic structure of the species using DNA sequence data (fragments of the COI and 28S genes). In particular, we looked for evidence that might indicate the presence of separate species in the North and South Island populations, as in the mayfly genera *Rallidens* and *Ichthybotus* (Hitchings et al. 2015). Cryptic species provide challenges for the accurate assessment of biodiversity and if unrecognised 'may lead to erroneous inferences in conservation' (Leys et al. 2016). The overarching purpose of this research was therefore to generate information that would aid conservation managers to protect a unique freshwater species and, in doing so, draw attention to freshwater invertebrate conservation in New Zealand.

Methods

Geographic distribution

Historical collection records for *Siphlaenigma janae* were obtained from national specimen collections, from State of the Environment monitoring data (1995–2011; MFE 2015) and from published literature (Penniket 1962; Towns 1978; Hitchings 2001; Hitchings et al. 2015). National specimen collections accessed included the New Zealand Arthropod Collection, the Auckland, Canterbury and New Zealand (Te Papa) Museum Collections and the Purdue Entomological Research Collection (Indiana, USA). Material in the Canterbury Museum, which held the largest number of specimens, was inspected to verify identifications.

In addition, a series of specimens were obtained in 2013–2016 as part of an extensive nationwide light trapping survey for mayflies and stoneflies (see Winterbourn et al. 2017). Also, during December 2016–January 2017 a number of sites were visited where *S. janae* had been previously found on the South Island's West Coast, and in the Northland and Taupo regions of the North Island. The primary purpose of sampling the 2016–2017 sites was to obtain fresh specimens for DNA analysis, and confirm present-day occurrence at these locations. Distribution data based on all confirmed or trusted records were plotted on a map (Figure 5.1) and a catalogue of collection details was generated (Supplementary Table S5.1).

Body size variability

Forewing length of all imaginal and subimaginal specimens present in the Canterbury Museum collection, and specimens taken in the 2013–2016 light trapping survey of the country, was measured to the nearest 0.1 mm with a linear micrometer inserted into a binocular microscope. Body lengths of final instar nymphs in the Canterbury Museum collection, and in collections made in the Pukenui Forest in Northland, were measured in the same way. Because telescoping of abdominal segments can affect the reliability of body length measurements of mayfly nymphs, measurements were adjusted where necessary to enable direct comparisons to be made. Although head width is often used as an indicator of body size, it was not considered to be useful for nymphs of *S. janae*, which have narrow hypognathous heads. The collection locations of all specimens fell into three geographical groups: northern North Island, central North Island, and northern South Island.

Genetic structure

DNA sequences were obtained for 37 individuals from seven locations (see Figure 5.1) for genetic analysis, using the standard 'barcoding' fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene (Hebert et al. 2003). Sampling locations provided broad geographic coverage of the known distribution of *S. janae* and all available specimens (1–10 individuals per location) were used in the

analysis. DNA was extracted from three legs of each specimen with an Extract-N-Amp Tissue PCR Kit (Sigma–Aldrich), the legs being crushed and added to 25 µL Extraction Solution and 7.0 µL Tissue Preparation Solution, incubated in a thermocycler at 37 °C for 30–45 min, then 95 °C for 3 min, and 25 µL Neutralising Solution added. The barcode fragment of the COI gene was amplified using the universal primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). Polymerase chain reaction (PCR) amplifications were performed in a final volume of 15 µL containing 2x Kapa2G Robust HotStart ReadyMix (7.5 µL), 0.35 µM of each primer (1 µL), nanopure H_2O (5.5 µL) and 1.0 µL of template DNA (30–86 ng/µL). Thermal cycling parameters included an initial denaturation at 95 °C for 3 min, followed by 35 cycles at 95 °C for 15 s, 50 °C for 20 s, 72 °C for 30 s and a final 5 min extension at 72 °C. PCR products were purified with incubation in 2 µL ExoSAP-IT reagent and sequenced in forward and reverse directions, using the same PCR primers used for amplification, by MacroGen (Seoul, South Korea). In addition, for seven individuals representing the three distinct COI haplotype networks found (see Results), the D2 fragment of the 28S nuclear gene was amplified with the primer pair 28Sa (5'-GACCCGTCTTGAAACACGGA-3') (Nunn et al. 1996) and LSUR (5'-GCTACTACCACCAAGATCTGCA-3') (Rix et al. 2008), using the same PCR, cleaning and sequencing procedures described above. Forward and reverse sequences of both COI and 28S were aligned manually and trimmed to 658 and 833 base pairs (bp), respectively, using BioEdit Sequence Alignment Editor version 7.2.5 (Hall 1999) and deposited in GenBank (Benson et al. 2013) (Accession Numbers: COI MF668529–MF668565; 28S MF668243–MF668249).

Statistical analyses

In the body size study, nymphs from the three geographic groups (see above) were compared using one-way ANOVA, followed by Tukey's pairwise HSD tests to determine where differences lay. A t-test was used to compare forewing length of male and female *S. janae* in the full data set. In the genetic structure study, haplotype composition and a statistical parsimony network were identified using TCS version 1.21 (Clement et al. 2000). Evolutionary relationships were then constructed with a Median-Joining network (Bandelt et al. 1999) (Epsilon = 0) in PopART version 1.7 (Leigh & Bryant 2015). Genetic distance within and among the seven populations was estimated in MEGA 7.0 (Kumar et al. 2016) using the uncorrected *p*-distance method (see Collins et al. 2012), and also with the Kimura two-parameter (K2P) model (Kimura 1980) to facilitate comparison with previously published studies. Our pairwise distance estimates employed the best-fitting nucleotide substitution model, a general time-reversible model with gamma-distributed among-site rate variation and invariant sites (GTR +G +I; shape parameter 0.4453; bootstrap = 2000) which was calculated in MEGA 7.0 and selected using the corrected Akaike Information Criterion (AICc, Akaike 1973). Genetic diversity indices were estimated with Nei's gene (Hd) and nucleotide diversity (π) (Nei 1987) in Arlequin version 3.5.1.3 (Excoffier & Lischer 2010). An Analysis

of Molecular Variance (AMOVA; 1000 permutations) was also run in Arlequin to examine the biogeographical effect on genetic variance using all data grouped as one 'New Zealand' dataset, followed by pairwise Φst population differentiation values. While an aim of the study was to assess whether sibling species may exist in the North and South Islands, constructing AMOVA groupings in this manner was not appropriate as only one South Island population was sampled (see Fitzpatrick 2009).

Results

Geographic distribution

Siphlaenigma janae has a fragmented distribution and has now been recorded from 42 locations ranging from Omahuta Forest in the north of the North Island to the vicinity of Lake Brunner in the northwest of the South Island (Figure 5.1, Supplementary Table S5.1). Collection localities ranged from 10 to 730 m above sea level (m a.s.l.). The earliest records of the species came from a low-gradient, forested tributary of the Arnold River in the Buller district of the South Island (Penniket 1962). This small stream (the type locality) no longer exists as the surrounding land has been drained and converted to pasture. There are also a number of other historic records in the vicinity, and west of Lake Brunner, but these localities have now been converted to exotic plantation forest, some of which have been logged and converted to pasture.

Whether *S. janae* still inhabits any of these streams is not known. However, specimens have been collected repeatedly from streams draining the Reefton Saddle, particularly O'Malley Creek. Nymphs have also been reported from coastal streams near Punakaiki and Karamea (Hitchings 2001; Hitchings et al. 2015) and further inland from Claybank Creek near Murchison (Towns 1978). However, the Karamea and Murchison specimens could not be found in their respective museum collections for validation, and no nymphs were found in Claybank Creek despite extensive searching in December 2016. Nevertheless, in December 2016 specimens were collected from O'Malley Creek (Reefton Saddle), which is currently the only South Island location where *S. janae* is definitely known to occur.

In the North Island *S. janae* has been recorded from Northland, the Waitakere, Hunua and Coromandel Ranges, and various locations across the central North Island from Rotorua and Waikaremoana in the east to inland Taranaki in the west. Specimens recorded in national State of the Environment monitoring programmes, and specimens collected in mayfly surveys during 2011–2017 (S. Pohe, unpubl. data), have expanded the known distribution of *S. janae* to parts of Northland, Auckland, Bay of Plenty, Hawkes Bay, Whanganui and the Rotorua-Taupo regions. Specimens reported from Waipoua (Northland) and Whatawhata (Waikato) by Hitchings (2001) and Hitchings et al. (2015), and present in the Canterbury Museum collection, were misidentified. However, *S. janae* is known to occur in these general localities.

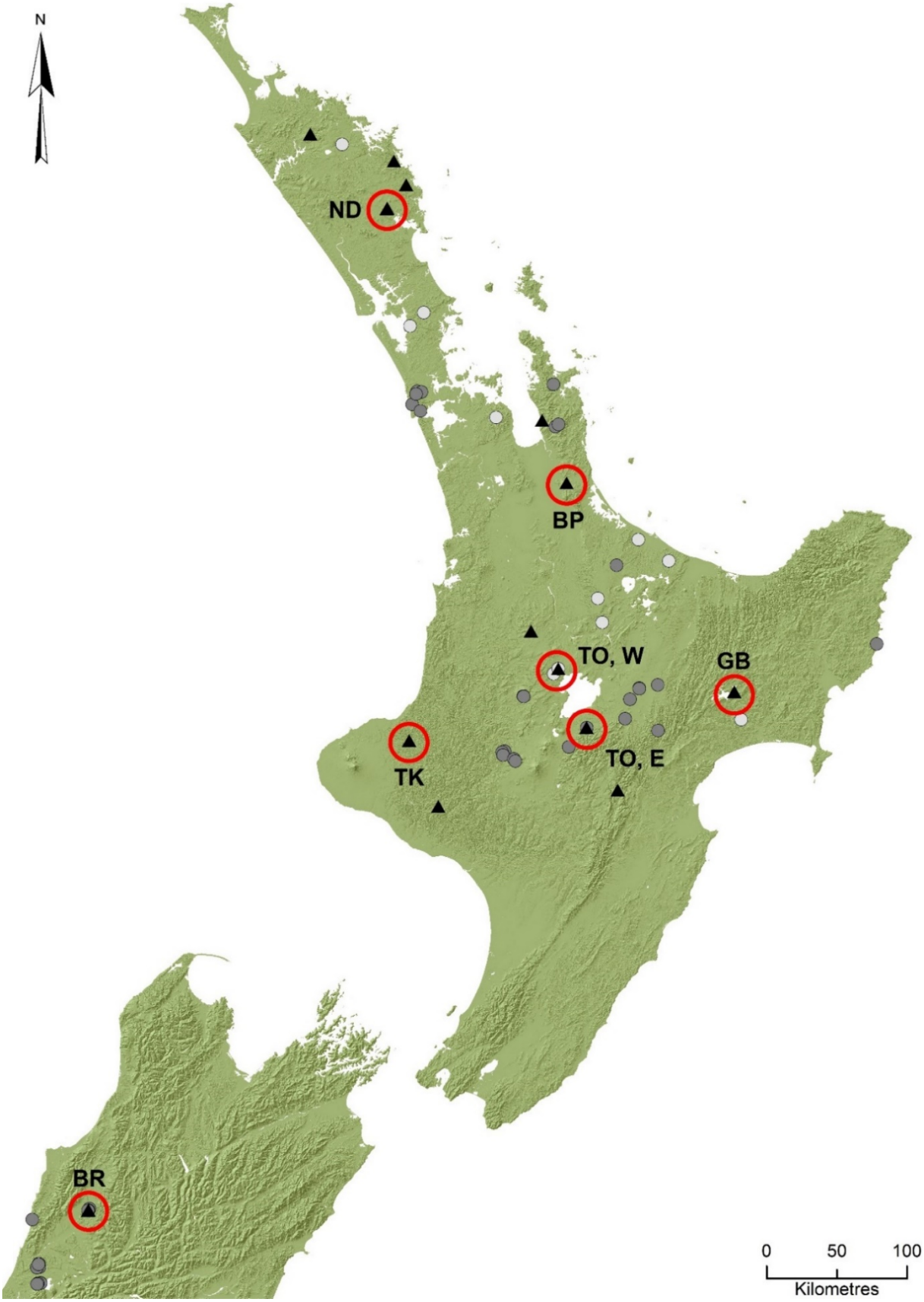

Figure 5.1 Distribution records for *Siphlaenigma janae*. Dark-grey points indicate locations of specimens held in national collections (1960–2003), light-grey points indicate locations of specimens recorded by State of Environment monitoring (2005–2010) and black triangles indicate locations of specimens collected by the authors (2011–2017). Red circles with corresponding codes indicate locations for which DNA analysis was undertaken in this study.

Body size variability

Body length measurements were made on 82 final instar nymphs, 31 from the South Island and 51 from the North Island. They formed three discreet geographical groups; one in the north of the North Island, one in the central North Island and one in the northwest of the South Island (Table 5.1). Although body size of final instar nymphs varied considerably within groups it differed significantly among them and between all pairs of groups (ANOVA, $F = 37.68$, $P < 0.0001$; Tukey's HSD tests, $P < 0.01$). Mean body length was shortest in the most northern group and longest in the central North Island.

Table 5.1 Body lengths of final instar nymphs of *Siphlaenigma janae* collected in three geographical areas of New Zealand.

	Northern North Island	Central North Island	Northern South Island
Number of specimens	18	33	31
Mean length ± SEM (mm)	5.7 ± 0.13	7.4 ± 0.08	6.8 ± 0.15
Maximum length (mm)	7.0	8.7	8.8
Minimum length (mm)	5.0	6.5	5.3

Twenty-five winged imagos and subimagos were available for measurement. The two northernmost specimens, a male and a female from Pukenui Forest near Whangarei, had forewing lengths of 5.9 mm and were the smallest individuals measured. Forewings of males from the central North Island and South Island ranged in length from 7.0–7.3 mm and 6.5–8.0 mm, respectively, whereas those of females ranged from 7.5–8.2 mm (central North Island) and 7.3–8.5 mm (South Island). Overall, females had significantly longer wings than males (t-test, $P < 0.05$).

Genetic structure

Sequence data for the standard barcode fragment of the mitochondrial COI gene were obtained from 37 *S. janae*, collected at seven locations across New Zealand. There was no evidence of saturation in the data. Mean nucleotide composition was T = 40.6%, C = 15.4%, A = 27.3% and G = 16.7% and of the 658-bp positions, 64 were variable and 56 parsimony-informative.

Overall nucleotide diversity (π) was 0.033 and from the 37 sequences generated, 28 haplotypes were identified, 22 of which were unique. For the three locations that had ten individuals (Northland, Taranaki, Reefton Saddle), haplotype diversity (Hd) within the populations was 0.93, 1.0 and 0.76, respectively. Evolutionary relationships estimated using a Median-Joining model produced a multi-modal 'long-chain' haplotype network (Figure 5.2). When the sequences were evaluated with TCS software they formed three distinct parsimony networks (= haplogroups) that were separated by at least 11 steps and could not be joined with 95% confidence. Haplogroup A comprised all ten individuals from Northland, the sole individual from the northeastern North Island (Waitawheta) and two of the ten individuals from Taranaki. Haplogroup B comprised 14 individuals, all from the central North Island (Taupo,

Waikaremoana and Taranaki) and Haplogroup C, all ten individuals from the northwestern South Island (Reefton Saddle).

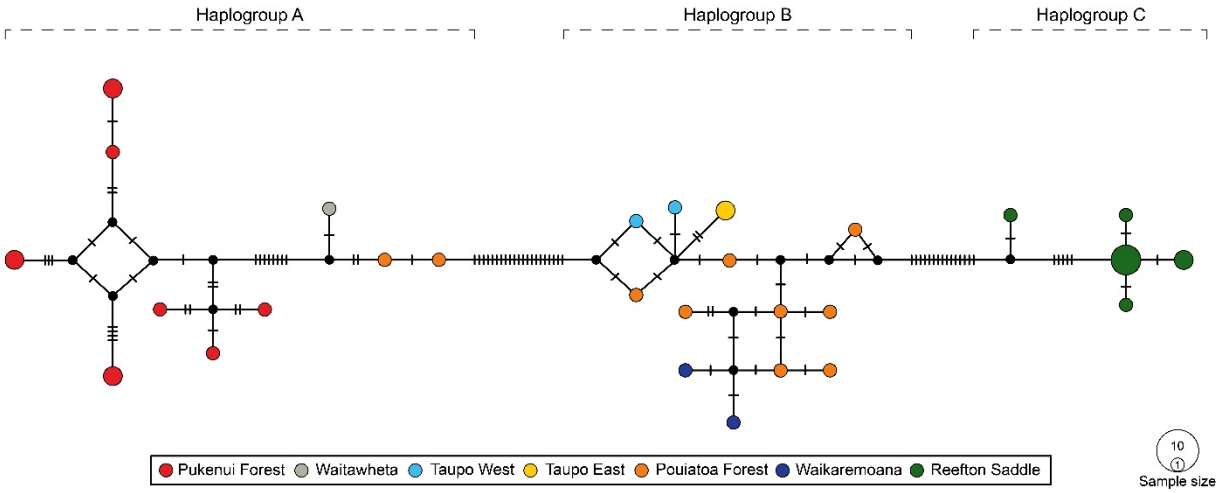

Figure 5.2 Median-Joining network for *Siphlaenigma janae* based on the barcoding fragment of the COI gene. Coloured points represent different sampling locations and the size of the points is relative to the frequency of the haplotypes. Black points indicate hypothetical haplotypes and hatch marks on the branches indicate the number of mutational steps.

No sharing of haplotypes between the seven locations was found, and while some within-location haplotype sharing was observed in Northland (Pukenui Forest) and in the South Island at Reefton Saddle (O'Malley Creek), the Taranaki (Pouiatoa Forest) haplotypes were all unique. Overall mean *p*-distance of the 28 haplotypes was 3.1% (3.4% K2P), means between groups ranged from 0.5–5.4% (0.5–6.4% K2P) and within-groups was 0.3–1.7% (0.3–1.9% K2P) (Table 5.2). Maximum genetic *p*-distance was 6.1% (7.3% K2P), between individuals from the northernmost and southernmost sampling locations, and maximum within-group distances ranged from 0.3–4.3% (0.3–4.8% K2P). AMOVA indicated that overall genetic differentiation in the population was significant ($\Phi st = 0.7529$, $P < 0.001$) with 75.3% of the genetic variation among populations and 24.7% within populations. In addition, significant mean pairwise differences were found between most populations, particularly between those with ten individuals each (Reefton Saddle and Northland, Reefton Saddle and Taranaki, Northland and Taranaki, $P < 0.001$ for each).

Sequences of the 28S nuclear gene were obtained for seven individuals representing the three distinct COI haplotype networks found. All seven sequences were identical (833-bp).

Table 5.2 Estimated evolutionary divergence of *Siphlaenigma janae*, based on a 658-bp fragment of the COI barcoding gene, presented as uncorrected *p*-distance (%). Between-group mean and maximum distances are presented above (blue text) and below the diagonal, respectively. Within group mean distances are presented on the diagonal (bold values). See Supplementary Table S5.2 for individual haplotype *p*-distances and standard error estimates. *n* = number of individuals, Hap = number of haplotypes. Abbreviations in parentheses follow locality codes of Crosby et al. (1998).

	n	Hap	(ND)	(BP)	(TK)	(TO, E)	(TO, W)	(GB)	(BR)
Pukenui Forest (ND)	10	7	**1.1**	2.0	4.0	4.2	4.4	4.4	5.4
Waitawheta (BP)	1	1	2.3	-	3.1	3.7	3.7	3.7	4.9
Pouiatoa Forest (TK)	10	10	5.0	4.0	**1.7**	1.3	1.2	1.3	3.7
Taupo East (TO, E)	2	1	4.4	3.7	3.7	-	0.5	0.6	3.3
Taupo West (TO, W)	2	2	4.9	3.8	3.8	0.5	**0.3**	0.8	3.8
Waikaremoana (GB)	2	2	4.7	3.7	4.0	0.6	0.8	**0.3**	3.5
Reefton Saddle (BR)	10	5	6.1	5.2	5.2	3.5	4.0	3.8	**0.5**

Discussion

Distribution and genetic structure

Siphlaenigma janae, the sole representative of the family Siphlaenigmatidae, is reported from 22 new localities within New Zealand, increasing the total number of records to 42. In particular, recent nationwide light trapping combined with benthic sampling of adjacent streams has extended the known distribution of the species in the northern and central North Island (Figure 5.1). However, the species appears to be absent from a number of South Island localities where it was historically recorded, notably at and around the type locality in the Buller district, which is likely a result of land-use changes. *Siphlaenigma janae* has not been found south of Lake Brunner in the South Island and there is a considerable distributional gap in the North Island, south of Hawkes Bay and Taranaki. The absence of records from the southern North Island is difficult to explain since suitable habitat would appear to be present in the foothills of the Tararua, Ruahine and Rimutaka ranges. The current distribution gap may simply be due to a lack of sampling effort. Further searches for *S. janae* in these areas, and in the Nelson and Buller regions (South Island), are warranted.

We found the typical habitat of nymphs to be low-gradient slow flowing sections of small streams with pools, in and alongside native forest (Figure 5.3). Throughout our surveys nymphs were not found in swift water, and in the Waitakere River Towns (1978) reported that nymphs were often associated with clumps of algae and the overhanging herb *Elatostema rugosum*. Most streams from which we collected *S. janae* were densely forested, often with steep banks that created very low-light conditions. In a few exceptions, where streams emerged into forest margins (including roadsides), nymphs were collected from vertical streambanks (i.e. clinging to the vertical banks) or from terrestrial vegetation trailing in the

water. It may be that *S. janae* nymphs are to some degree negatively phototactic and that vertical banks and trailing vegetation reduce light to acceptable levels in relatively open situations.

Nucleotide diversity (π), one measure of genetic variability, was higher in *S. janae* (0.0334) than reported in several other insect studies including that of the New Zealand giraffe weevil, *Lasiorhynchus barbicornis* (0.0121; Painting et al. 2017), which has a comparable North Island distribution, a Malaysian blackfly *Simulium tani* (0.0089; Low et al. 2014) and the neotropical butterfly *Heliconus erato* (0.022; Counterman et al. 2010). Our body size study of final instar nymphs also indicated considerable variation in regional populations, and no significant latitudinal or altitudinal trends were found. However, because precise body length measurements of preserved mayflies are difficult to obtain due to telescoping, and that material was available from only a small number of populations, regional patterns in nymphal size may have been obscured. Nevertheless, adult body size, measured as forewing length, indicated specimens from the far north of the country were the smallest recorded, a pattern also found in the New Zealand stonefly *Stenoperla prasina* (Winterbourn et al. 2017).

The 37 sequenced individuals of *S. janae* from the seven localities in the genetic study revealed 28 haplotypes, 22 of which were unique. They formed three haplogroups centred in the northern and central North Island, and in the northwest of the South Island. The North Island haplogroups are essentially congruent with McGlone's (1985) centres of plant endemism and likely represent areas of survival and/or evolution during the last ice age. A restricted Pleistocene distribution with important northern refugia from which range expansion followed glacial retreat has also been proposed for *L. barbicornis* (Painting et al. 2017). Similarly, Smith et al. (2006) suggested that the levels of sequence divergence found in the mayfly *Acanthophlebia cruentata* could be interpreted as the result of Pleistocene divergence in multiple refugia leading to the evolution of regionally unique haplotypes. The southern limit of the northern haplogroup of *S. janae* near the base of the Coromandel Peninsula was coincident with the 'Kauri Line' of Wagstaff and Clarkson (2012), which was also the southern limit of genetic clades of cicada species (Ellis et al. 2015). Together, these studies provide strong evidence that Northland was a significant faunal refuge in the Pleistocene from which subsequent southern dispersal of insects has occurred.

Figure 5.3 Typical native forest stream habitat of nymphs of *Siphlaenigma janae* in the Pukenui Forest, Northland. **A,** Pool below a small waterfall, with depositional zones and overhanging streamside vegetation. **B**, Low-gradient slow-flowing stream section (a run) below a gravel-bar riffle, with vertical and undercut streambanks.

As in *L. barbicornis* (Painting et al. 2017) a high proportion of unique haplotypes was found in *S. janae*, a condition which suggests that populations have continued to evolve in relative isolation. Such a scenario is consistent with the findings of McCulloch et al. (2010) where haplotype sharing was more common among populations of macropterous than apterous genera of New Zealand stoneflies (Plecoptera). The multi-modal 'long-chain' haplotype network found in *S. janae* is characteristic of old

populations of constant size (Sekiné et al. 2017), and in conjunction with its high haplotype diversity and predominance of unique haplotypes, it suggests that populations have evolved in moderate isolation. As dispersal of this small, weak-flying insect is probably limited, genetic variation has likely accrued locally by combinations of drift, selection and mutation (McLean et al. 2008). In contrast, the large New Zealand stonefly *S. prasina* is a strong-flying species that has few unique haplotypes, restricted to the far north of the country (Winterbourn et al. 2017).

Does genetic distance among the three haplogroups of *S. janae* indicate the presence of cryptic species? Comparative data obtained by Ball et al. (2005) indicated that the mean divergence (K2P distances) of COI barcode fragments between more than 200 species pairs of North American Ephemeroptera (11 families) was 18%. Only two interspecific distances (*Caenis* 3.3%, *Baetisca* 3.8%) were smaller than the maximum *p*-distance of 6.1 % (7.3% K2P) we obtained for *S. janae*. In two more recent studies that used uncorrected *p*-distances of the COI barcode fragment, Williams et al. (2006) found haplogroup diversity of 0.2–3% within, and 8–19% among groups of *Baetis rhodani* in Great Britain, Scandinavia and Western Europe, and Ståhls and Savolainen (2008) found 0.3–1.4% within and 13.1–16.5% between haplogroups of *B. vernus* in northern Europe. These large genetic distances between haplogroups led Williams et al. (2006) and Ståhls and Savolainen (2008) to conclude there was strong evidence for the presence of cryptic species. In contrast, the smaller maximum distances found in *S. janae* (0.5–6.1% [0.5–7.3% K2P]) are less convincing. However, discrimination of species depends on how species are defined. If species are separately evolving metapopulation lineages as proposed by de Queiroz (2007) as the basis of the 'unified species concept', then the three haplogroups identified among *S. janae* support the existence of three separate species. Because our sequences for the D2 region of the 28S nuclear gene were all identical it is tempting to conclude that the three haplogroups represent a single species. However, ribosomal 28S is generally viewed as being too highly conserved to enable resolution at the species level (Sonnenberg et al. 2007; Carew & Hoffmann 2015; Guerra et al. 2016), although some species of caddisflies (Kjer et al. 2014), beetles (Monaghan et al. 2005) and fish (Sonnenberg et al. 2007) have been successfully resolved using the 28S gene. At present, *S. janae* populations might best be regarded as incipient species given their differentiation is based on limited sequence data, sample sizes are not large and morphological assessments of the geographical groups have not been made.

Conservation

Freshwater invertebrates are under-represented in assessments of conservation status and often neglected in targeted aquatic conservation efforts (Collier et al. 2016). There is a clear need for more data on the abundance, distribution and diversity of taxa (Collier et al. 2016; Gerbeaux et al. 2016) and an improved understanding of ecology and genetic diversity is needed to conserve biodiversity and manage natural systems (Stauffer-Olsen et al. 2017). The motivation for the present study was to

address some of these needs with respect to the taxonomically unique New Zealand mayfly *Siphlaenigma janae*. However, only a handful of adult specimens are held in national collections, limiting the advancement of any taxonomic research, and only nine winged adults were taken by light traps in our recent nationwide survey, compared to over 20,000 other mayfly individuals. In addition, it is a difficult insect to study because of its small size, low densities throughout its range, elusive adult life stage and because it has a nymphal habitat (low-gradient sections of forested streams) that is seldom sampled in biological monitoring programmes or more general aquatic ecology studies.

Currently, *S. janae* has a threat classification of Nationally Vulnerable (Grainger et al. 2014), a status which we believe is appropriate given that the nymphal habitat is under pressure from human development, the genetic distinctiveness of regional populations, and the limited area and size of the fragmented populations, particularly those in the South Island. Nevertheless, our recent surveys increased knowledge of its distribution with six new locations in the northern North Island, and five new (and two reconfirmed) locations in the central North Island. Our surveys also revealed that the type locality, 'Baxter's Creek' near Lake Brunner, has been lost due to conversion to pasture. Other South Island streams where it has been found in the past are now also in pasture, or converted to plantation forest, so it is not known whether extant populations have endured.

Within the limits of its known distribution, South Island populations of *S. janae* appear to be most at risk due to land-use activities that include forestry, farming and possibly mining. There is a clear need to ensure that forested reserves encompassing low-gradient streams, particularly where the species is known to occur, have active conservation management. Implementation of assessments to gauge potential threats to stream habitat, and additional surveys to look for other southern populations, would be positive first steps. In particular, sites in and adjoining the Kahurangi National Park, Paparoa National Park, southern Victoria Forest Park (Tawhai Forest) and the Card Creek Ecological Area would be appropriate. Because the South Island population on the Reefton Saddle represents a distinct haplogroup not found elsewhere, the need for it to be protected is all the greater. Similarly, consideration of the conservation value of the distinctive northern haplogroup is warranted, which includes the historically important population in the Waitakere Ranges (Towns 1978) that has not been recorded since 1993. In contrast, *S. janae* appears to have numerous viable populations in conservation-managed native forest streams in the central North Island. However, there is a need for surveys in the Waikato, Wellington, Wairarapa and Marlborough regions where *S. janae* might be expected to occur, but currently have no records. Furthermore, surveys attempting to confirm presence at sites of historical records in the vicinity of the type locality (near Lake Brunner), and in the Nelson region, would be valuable.

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Supplementary material

Table S5.1 Catalogue of collection records for *Siphlaenigma janae**. Locations in **bold** indicate specimens for which DNA analyses were undertaken in this study. Location abbreviations in parentheses follow Crosby et al. (1998). Repository abbreviations are: RC = Regional Council, Pohe Coll. = Stephen R. Pohe Collection, Cant. Mus. = Canterbury Museum, NZAC = New Zealand Arthropod Collection, FAMU = Florida A & M University, Mus. of N.Z. = Museum of New Zealand, PERC = Purdue Entomological Research Collection. Coordinates of museum collection records are approximations from label data.

Location	Lat. °S; Long. °E	Collectors and dates	Repository
Waikoropupu Stm, Omahuta (ND)	35.2331; 173.6525	Tanya L. Cook; 2001	Pohe Coll.
Waitangi River, Waimate North (ND)	35.2963; 173.9016	Northland RC; 2008	Pohe Coll.
Punaruku Stm, Punaruku (ND)	35.3978; 174.3111	Stephen R. Pohe; 2013	Pohe Coll.
Te Oriwa Stm, Whananaki South (ND)	35.5506; 174.4098	Stephen R. Pohe; 2013	Pohe Coll.
Pukenui Stm, Whangarei (ND)	35.7019; 174.2645	Stephen R. Pohe; 2011–2013, 2017	Pohe Coll.
Awarere Stm, Warkworth (AK)	36.3700; 174.5676	Auckland RC; 2009	
Omaumau River, Mt Auckland (AK)	36.4577; 174.4603	Auckland RC; 2008, 2010	
Awaroa Stream, Whitianga (CL)	36.8105; 175.6149	Brian J. Smith; 1999	Cant. Mus.
Waitupu Stm, Waitakere Ranges (AK)	36.8763; 174.5279	Arnold H. Staniczek; 1993	Cant. Mus.
Swanson Stm, Waitakere Ranges (AK)	36.8796; 174.5604	John A. McLean; 1966	NZAC
Cascade Stm, Waitakere Ranges (AK)	36.8932; 174.5185	David R. Towns; 1976	NZAC
Kitekite Falls, Waitakere Ranges (AK)	36.9608; 174.4890	Towns & Hayward; 1976	
Karamatura Stm, Waitakere Ranges (AK)	37.0020; 174.5556	Towns & Towns; 1977	FAMU
Mangatawhiri River, Hunua Ranges (AK)	37.0325; 175.1626	Auckland RC; 2010	
Te Puru Stm, Te Puru (CL)	37.0450; 175.5337	Stephen R. Pohe; 2013	Pohe Coll.
Kauaeranga River, Thames (CL)	37.0667; 175.6659	Towns & Hayward; 1977	
Whangaiterenga Stm, Thames (CL)	37.0830; 175.6430	Arnold H. Staniczek; 1993	Cant. Mus.
Waitawheta River, Waitawheta (BP)	37.4415; 175.7453	Stephen R. Pohe; 2013	Pohe Coll.
Waiari Stm, Te Puke (BP)	37.7906; 176.3384	Bay of Plenty RC; 2005	
Pungarehu Stm, Te Puke (BP)	37.9195; 176.5935	Bay of Plenty RC; 2005	
Mangorewa River, Rotorua (BP)	37.9600; 176.1702	Terry R. Hitchings; 1995	Cant. Mus.
Mokaihaha Stm, Rotorua (BP)	38.1766; 176.0279	Environment Waikato RC; 2008	
Tahunaatara Stm, Rotorua (BP)	38.3310; 176.0700	Environment Waikato RC; 2008	
Unnamed stream, Tolaga Bay (GB)	38.3870; 178.3164	Graeme J. Penney; 1996	Cant. Mus.
Okahukura Stm, Tokoroa (TO)	38.4008; 175.4925	Stephen R. Pohe; 2014	Pohe Coll.
Waihora Stm, Taupo (TO)	38.6376; 175.7237	Stephen R. Pohe; 2017	Pohe Coll.
Waihora Stm, Taupo (TO)	38.6377; 175.7245	Environment Waikato RC; 2008	
Otupoto Stm, Taupo (TO)	38.6708; 175.6873	Environment Waikato RC; 2008	
Ngapuketurua, Kaingaroa Forest (TO)	38.7206; 176.5418	M. M. Neill; 1965	Mus. of N.Z.
Aniwaniwa Stm, Waikaremoana (GB)	38.7434; 177.1726	Stephen R. Pohe; 2014	Pohe Coll.

Otamatea River, Kaingaroa Forest (TO)	38.7462; 176.3913	Crawford & Hitchings; 1998, 1999	Cant. Mus.
Rangitaiki River, Kaingaroa Forest (TO)	38.8186; 176.3199	William J. Crawford; 1997	Cant. Mus.
Taringamotu River, Oruaiwi (TO)	38.8228; 175.4443	P. Aston; 1963, Penniket; 1966	Cant. Mus.
Taringamotu River, Oruaiwi (TO)	38.8228; 175.4443	P. Aston; 1969	PERC
Te Iringaowhare Stm, Wairoa (GB)	38.9194; 177.2401	Hawke's Bay RC; 2009	
Taharua River, Kaingaroa Forest (TO)	38.9458; 176.2856	William J. Crawford; 2002	Cant. Mus.
Mangakowhitiwhiti Stm, Turangi (TO)	39.0110; 175.9706	Stephen R. Pohe; 2016	Pohe Coll.
Mangakowhitiwhiti Stm, Turangi (TO)	39.0112; 175.9709	William J. Crawford; 2003	Cant. Mus.
Waipunga River, Tarawera (TO)	39.0133; 176.5592	William J. Crawford; 2002	Cant. Mus.
Waitara River, Pouiatoa Forest (TK)	39.1234; 174.5119	Stephen R. Pohe; 2014	Pohe Coll.
Waikoko Stm, Rangipo (TO)	39.1383; 175.8305	William J. Crawford; 2002	Cant. Mus.
Tupapakurua Stm, National Park (TO)	39.1773; 175.3006	William J. Crawford; 2003	Cant. Mus.
Retaruke River, National Park (TO)	39.1858; 175.2812	Terry R. Hitchings; 1998	Cant. Mus.
Retaruke River, National Park (TO)	39.2037; 175.2888	William J. Crawford; 2003	Cant. Mus.
Waimarino Stm, National Park (TO)	39.2270; 175.3799	William J. Crawford; 2002	Cant. Mus.
Makomiko Stm, National Park (TO)	39.2403; 175.3920	William J. Crawford; 2002	Cant. Mus.
Mangataramea Stm, Timahanga Stn (TO)	39.4061; 176.2452	Stephen R. Pohe; 2014	Pohe Coll.
Waitotara River, Taumatatahi (RI)	39.5430; 174.7630	Stephen R. Pohe; 2014	Pohe Coll.
Jinks Creek, Reefton Saddle (BR)	42.1343; 171.8231	Terry R. Hitchings; 1997	Cant. Mus.
O'Malley Creek, Reefton Saddle (BR)	42.1450; 171.8156	Terry R. Hitchings; 1997	Cant. Mus.
O'Malley Creek, Reefton Saddle (BR)	42.1459; 171.8140	Harding, Winterbourn, Pohe; 2016	Pohe Coll.
O'Malley Creek, Reefton Saddle (BR)	42.1461; 171.8108	Michael J. Winterbourn; 2007	
O'Malley Creek, Reefton Saddle (BR)	42.1468; 171.8120	Arnold H. Staniczek; 1993	Cant. Mus.
Deverys Creek, Barrytown (BR)	42.1983; 171.3291	Terry R. Hitchings; 2000	Cant. Mus.
Baxter's Creek, Kokiri (BR)	42.4871; 171.3761	John G. Penniket; 1960	Cant. Mus.
Baxter's Creek, Kokiri (BR)	42.4871; 171.3761	John G. Penniket; 1960	NZAC
Baxter's Creek, Kokiri (BR)	42.4871; 171.3761	John G. Penniket; 1966	PERC
Arnold Creek, Kokiri (BR)	42.5052; 171.3817	John G. Penniket; 1966	Cant. Mus.
Maori Gully Creek, Kokiri (BR)	42.5084; 171.3597	Terry R. Hitchings; 1993	Cant. Mus.
Pigeon Creek, Lake Brunner (BR)	42.6079; 171.3912	Terry R. Hitchings; 1998	Cant. Mus.
Niagara Creek, Lake Brunner (BR)	42.6085; 171.3656	Terry R. Hitchings; 1998	Cant. Mus.
Eastern Hohonu River, Lake Brunner (BR)	42.6129; 171.3593	Terry R. Hitchings; 1998	Cant. Mus.

* Records of *S. janae* from Murchison (Towns 1978) and Karamea (Hitchings 2001; Hitchings et al. 2015) could not be validated and are not included in this list.

Table S5.2 The number of base differences per site (%) between the 28 haplotypes of *Siphlaenigma janae* found, based on a 658-bp fragment of the COI barcoding gene, are presented below the diagonal. Standard error estimates (%) are shown above the diagonal (blue values). Sample abbreviations in parentheses follow locality codes of Crosby et al. (1998).

Sample	1630	1687	473	474	475	476	478	375	379	385	387	390	499	500	543	6513	6623	6628	6676	6554	6555	6570	6589	6600	6531	3859	4115	4169
Pukenui Forest 1630 (ND)		0.4	0.5	0.3	0.5	0.4	0.3	0.9	0.8	0.8	0.8	0.8	0.8	0.7	0.8	0.7	0.7	0.5	0.7	0.5	0.7	0.7	0.8	0.8	0.8	0.5	0.8	0.8
Pukenui Forest 1687 (ND)	1.4		0.4	0.4	0.4	0.1	0.4	0.9	0.9	0.9	0.9	0.8	0.8	0.8	0.8	0.8	0.8	0.6	0.8	0.6	0.8	0.8	0.8	0.8	0.8	0.5	0.8	0.8
Pukenui Forest 473 (ND)	1.5	1.1		0.4	0.4	0.4	0.4	0.9	0.9	0.9	0.9	0.8	0.8	0.7	0.7	0.8	0.8	0.6	0.8	0.6	0.8	0.8	0.8	0.8	0.8	0.5	0.7	0.8
Pukenui Forest 474 (ND)	0.6	1.4	1.2		0.5	0.4	0.3	0.9	0.9	0.8	0.9	0.8	0.7	0.7	0.7	0.7	0.7	0.5	0.7	0.5	0.8	0.7	0.7	0.7	0.8	0.5	0.7	0.8
Pukenui Forest 475 (ND)	1.5	1.4	1.2	1.5		0.4	0.4	0.9	0.9	0.9	0.9	0.8	0.8	0.8	0.7	0.8	0.8	0.6	0.8	0.6	0.8	0.8	0.8	0.8	0.8	0.6	0.8	0.8
Pukenui Forest 476 (ND)	1.2	0.2	0.9	1.2	1.2		0.4	0.9	0.9	0.9	0.9	0.8	0.8	0.8	0.7	0.8	0.8	0.6	0.8	0.6	0.8	0.8	0.8	0.8	0.8	0.5	0.8	0.8
Pukenui Forest 478 (ND)	0.5	1.2	1.4	0.5	1.4	1.1		0.9	0.9	0.8	0.9	0.8	0.7	0.7	0.7	0.7	0.7	0.5	0.7	0.5	0.7	0.7	0.7	0.7	0.7	0.5	0.7	0.7
Reefton Saddle 375 (BR)	5.3	6.1	5.9	5.6	5.6	5.9	5.5		0.1	0.2	0.2	0.4	0.7	0.7	0.7	0.7	0.7	0.9	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.9	0.7	0.7
Reefton Saddle 379 (BR)	5.2	5.9	5.8	5.5	5.5	5.8	5.3	0.2		0.2	0.1	0.4	0.7	0.7	0.7	0.7	0.6	0.8	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.7
Reefton Saddle 385 (BR)	5.0	5.8	5.9	5.3	5.6	5.6	5.2	0.3	0.2		0.2	0.4	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.7
Reefton Saddle 387 (BR)	5.3	6.1	5.9	5.6	5.6	5.9	5.5	0.3	0.2	0.3		0.4	0.7	0.7	0.7	0.7	0.7	0.9	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.7
Reefton Saddle 390 (BR)	4.3	5.0	4.9	4.6	4.6	4.9	4.4	1.1	0.9	1.1	1.1		0.7	0.7	0.6	0.6	0.6	0.8	0.6	0.7	0.6	0.6	0.6	0.6	0.7	0.7	0.6	0.6
Taupo West 499 (TO)	4.4	4.9	4.4	4.4	4.7	4.7	4.3	4.0	3.8	4.0	4.0	3.2		0.2	0.3	0.3	0.3	0.7	0.2	0.7	0.2	0.3	0.4	0.3	0.3	0.7	0.3	0.3
Taupo West 500 (TO)	4.1	4.6	4.1	4.1	4.4	4.4	4.0	4.0	3.8	4.0	4.0	3.2	0.3		0.3	0.3	0.3	0.7	0.2	0.7	0.2	0.3	0.4	0.3	0.3	0.7	0.3	0.3
Taupo East 543 (TO)	4.3	4.4	4.0	4.3	4.3	4.3	4.1	3.5	3.4	3.5	3.5	2.7	0.5	0.5		0.4	0.4	0.7	0.3	0.7	0.3	0.3	0.3	0.3	0.4	0.7	0.3	0.3
Pouiatoa Forest 6513 (TK)	4.0	4.7	4.3	4.0	4.6	4.6	3.8	3.5	3.4	3.5	3.5	2.7	0.8	0.8	0.9		0.3	0.7	0.3	0.7	0.3	0.2	0.3	0.2	0.3	0.7	0.3	0.3
Pouiatoa Forest 6623 (TK)	4.0	4.7	4.3	4.0	4.6	4.6	3.8	3.4	3.2	3.4	3.4	2.6	0.8	0.8	0.9	0.6		0.7	0.3	0.7	0.3	0.3	0.3	0.3	0.4	0.7	0.3	0.4
Pouiatoa Forest 6628 (TK)	1.8	2.3	2.1	2.1	2.4	2.1	2.0	5.2	5.0	4.9	5.2	4.1	3.8	3.5	3.7	4.0	3.8		0.7	0.1	0.7	0.7	0.8	0.7	0.8	0.3	0.7	0.8
Pouiatoa Forest 6676 (TK)	4.1	4.9	4.4	4.1	4.7	4.7	4.0	3.7	3.5	3.7	3.7	2.9	0.3	0.3	0.5	0.5	0.5	3.8		0.7	0.2	0.2	0.3	0.2	0.3	0.7	0.3	0.3
Pouiatoa Forest 6554 (TK)	1.7	2.4	2.3	2.0	2.6	2.3	1.8	5.0	4.9	4.7	5.0	4.0	3.7	3.4	3.5	3.8	3.7	0.2	3.7		0.6	0.7	0.7	0.7	0.7	0.3	0.7	0.7
Pouiatoa Forest 6555 (TK)	4.1	4.9	4.4	4.4	4.7	4.7	4.3	3.7	3.5	3.7	3.7	2.9	0.3	0.3	0.5	0.8	0.8	3.5	0.3	3.4		0.3	0.4	0.3	0.3	0.7	0.3	0.3
Pouiatoa Forest 6570 (TK)	4.0	4.7	4.3	4.0	4.6	4.6	3.8	3.5	3.4	3.5	3.5	2.7	0.8	0.8	0.6	0.3	0.6	4.0	0.5	3.8	0.8		0.1	0.1	0.3	0.7	0.2	0.2
Pouiatoa Forest 6589 (TK)	4.1	4.9	4.4	4.1	4.7	4.7	4.0	3.7	3.5	3.7	3.7	2.9	0.9	0.9	0.8	0.5	0.8	4.1	0.6	4.0	0.9	0.2		0.2	0.3	0.7	0.3	0.3
Pouiatoa Forest 6600 (TK)	4.1	4.9	4.4	4.1	4.7	4.7	4.0	3.7	3.5	3.7	3.7	2.9	0.6	0.6	0.8	0.2	0.5	4.1	0.3	4.0	0.6	0.2	0.3		0.2	0.7	0.3	0.3
Pouiatoa Forest 6531 (TK)	4.6	5.0	4.6	4.6	4.9	4.9	4.4	4.1	4.0	4.1	4.1	3.4	0.8	0.8	0.9	0.6	0.9	4.3	0.8	4.1	0.8	0.6	0.8	0.5		0.7	0.3	0.3
Waitawheta 3859 (BP)	1.7	2.1	2.0	2.0	2.3	2.0	1.8	5.2	5.0	4.9	5.2	4.1	3.8	3.5	3.7	3.7	3.5	0.5	3.8	0.6	3.5	3.7	3.8	3.8	4.0		0.7	0.7
Waikaremoana 4115 (GB)	4.3	4.7	4.0	4.0	4.6	4.6	4.1	3.8	3.7	3.8	3.8	3.0	0.8	0.8	0.6	0.6	0.9	4.0	0.8	3.8	0.8	0.3	0.5	0.5	0.6	3.7		0.2
Waikaremoana 4169 (GB)	4.3	4.7	4.3	4.3	4.6	4.6	4.1	3.5	3.4	3.5	3.5	3.0	0.8	0.8	0.6	0.6	0.9	4.0	0.8	3.8	0.8	0.3	0.5	0.5	0.6	3.7	0.3	

Chapter 6 – *Ecology and conservation of Isothraulus abditus*

"If you want to be a good archaeologist,

you've got to get out of the library."

Harrison Ford (as Indiana Jones), 2008.

Imago male of *Isothraulus abditus* (Ephemeroptera). Photo credit: Olivier Ball / Stephen Pohe Collection.

Chapter 6 preface

This chapter describes a study of the poorly known species *Isothraulus abditus* and has been published as Stephen R. Pohe, Michael J. Winterbourn, Sharyn J. Goldstien, Olivier J.-P. Ball and Jon S. Harding. 2019. Distribution, nymphal habitat, genetic structure and conservation of the New Zealand mayfly *Isothraulus abditus* (Insecta: Ephemeroptera) and a description of its subimago. New Zealand Journal of Zoology 46: 13–30. https://doi.org/10.1080/03014223.2018.1464034. The journal's style and conventions have been retained but the format of tables and figures have been adjusted to fit the broader thesis layout. The subimago description, presented as Appendix 1 in the publication, is included here after the Discussion. Supplementary material referenced in the text of this chapter is hyperlinked to the published online versions, and hyperlinks are also provided at the end of the chapter. I undertook the data collection and analyses, laboratory work and wrote the draft manuscript. My co-authors Dr Ball and Prof. Harding provided advice on study design and commented on the final draft of the manuscript. Dr Goldstein provided training of genetic techniques in the laboratory, assistance with the genetic analyses and interpretation and commented on drafts of the manuscript. Prof. Winterbourn provided advice on study design, assistance with genetic interpretations and manuscript writing, and commented on drafts of the manuscript.

Abstract

Distribution, nymphal habitat and genetic structure of the New Zealand mayfly *Isothraulus abditus* were investigated to improve our understanding of its ecology and conservation status. A description of the undescribed subimaginal stage is also provided. *Isothraulus abditus* has now been recorded from 55 North Island streams, 47 being new to published literature. Its distribution currently extends from Mangonui in Northland to Taumatatahi near Whanganui. Nymphs live in forested stream pools and backwaters containing organic detritus. Genetic structure of six populations was assessed using the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. Thirty-six haplotypes were identified from 123 individuals, and a maximum uncorrected genetic distance of 1.8% indicated that specimens belong to a single species. However, those within Northland showed some genetic differentiation. Restricted habitat, sparse distribution and some genetic differentiation of *I. abditus* suggest its conservation status should be classified as 'At Risk'.

Introduction

Mayflies (Ephemeroptera) are widely distributed in freshwaters throughout the world. In many streams and rivers they are among the most abundant benthic invertebrates and are an important food supply for fish and other predators (Hynes 1970). Mayflies can also be valuable indicators of organic enrichment as they are particularly sensitive to changes in water quality and the degradation of catchments (Towns

& Peters 1996). However, not all species are equally sensitive to different kinds of pollution (Gray & Harding 2012; Stark 1993) and much more knowledge of their distributions, habitat requirements and biology is needed if their role as ecological indicators is to be optimised. Because of their sensitivity to changes in habitat loss and land use, mayflies may also require conservation effort if viable populations of individual species are to be maintained.

Towns and Peters (1996) considered New Zealand's fringe-gilled mayfly *Isothraulus abditus* Towns and Peters, 1979 to be one of New Zealand's rarest mayflies. The specific name *abditus*, meaning hidden, refers to the biology of the nymphs (Towns & Peters 1979); a condition that has undoubtedly contributed to the perception of rarity. *Isothraulus abditus* was described from specimens collected in small streams of Auckland's Waitakere Ranges (Towns & Peters 1979) following a pioneering study of freshwater invertebrate communities by Towns (1976). Imagos (adults) of *I. abditus* are small insects with a body length of 6.7–7.9 mm and forewings 7.5–8.8 mm long. Distinctive features are the narrow, elongate male penes, which are at least half the length of the genital forceps, and a ventral egg guide (ovipositor) that extends the length of abdominal segment 8 in females. The subimaginal stage (subadult) has not been described, although Towns and Peters (1979) listed a female subimago among their paratype material from the Waitakere River. The nymph is distinctive, as the dorsal and ventral portions of each gill lamella are fringed with narrow finger-like lappets, some of which may be longer than the lamella itself.

Isothraulus abditus is the only known species in its genus and may have relatives in New Caledonia (Towns & Peters 1980, 1996). There are also numerous morphological similarities between *Isothraulus* and the tropical genus *Thraulus*, including the distinctive fringed gills of the nymph. Its closest relative in the New Zealand fauna is *Tepakia* (Towns & Peters 1996) and numerous morphological similarities have also been noted between *Isothraulus* and a third New Zealand genus, *Zephlebia*, to which *I. abditus* was initially referred (Towns 1976, 1978). A recent molecular study of world Leptophlebiidae based on *histone H3* and *28S ribosomal* DNA consistently grouped *Tepakia*, *Isothraulus* and *Zephlebia* together within a Palaeoaustral group of genera (O'Donnell & Jockusch 2008), but distant from *Thraulus*, suggesting that similarities between *I. abditus* and *Thraulus* spp. may result from convergence. Beet (2016) also reported that *Isothraulus* and *Zephlebia* grouped together in a phylogenetic analysis using the mitochondrial cytochrome *c* oxidase subunit I (COI) gene; *Tepakia* was not included in her study.

Published records identified *I. abditus* in the vicinity of Auckland and on nearby Great Barrier Island (Towns 1987; Towns & Peters 1979, 1996), and in the adjacent regions of Northland and Coromandel (Collier 1995; Hitchings 2001; Hitchings et al. 2015; Pohe et al. 2018a). It was also thought to be present on Little Barrier Island, which lies off the Auckland coast (personal communication from T.K. Crosby to D.R. Towns, cited in Towns and Peters 1979). *Isothraulus abditus* was listed as of potential conservation

interest by Collier (1992, 1993) due to its known distribution being restricted to two ecological regions (*sensu* McEwen 1987), Auckland and Coromandel. Towns (1987) found numerous nymphs in an isolated pool filled with leaves and twigs on Great Barrier Island and other nymphs in first-order streams with little discernible water movement; stream habitats to which little ecological attention has been paid. These observations led him to suggest that intermittent streams might provide the most common habitat for nymphs, despite water in isolated, leaf-filled pools often providing poor-quality conditions for most mayfly nymphs. This is because such habitats probably develop depleted levels of dissolved oxygen and higher water temperature than the mainstream. However, no further work has been done to corroborate the observations of Towns (1987) that intermittent streams are indeed common habitats of *I. abditus* nymphs, or whether such environments are able to support them.

Genetic structure, an important tool in the management of species of conservation interest, has only been investigated in three species of New Zealand mayfly. *Coloburiscus humeralis* (Walker, 1853) showed minimal allelic differences that appeared unrelated to geographic proximity across its New Zealand distribution (Hogg et al. 2002). However, *Acanthophlebia cruentata* (Hudson, 1904) and *Siphlaenigma janae* Penniket, 1962 showed moderate to high levels of genetic differentiation among some North Island populations, and both species had unique haplotypes in the far north of New Zealand (Pohe et al. 2018b; Smith & Collier 2001; Smith et al. 2006).

The aims of the present study were to (1) update the known distribution of *I. abditus* based on extensive surveys undertaken by the authors, and records of its occurrence in published and unpublished sources, including museum specimen collections; (2) investigate habitat preference of the nymphs through a quantitative benthic survey, with particular attention to the association of nymphal occurrence and environmental conditions; (3) investigate the genetic structure of the species using DNA sequence data of the mitochondrial COI gene fragment to examine genetic structure within and between localities; and (4) assess the conservation status and requirements of the species using the new information we present. Based on material collected by the lead author during light trapping surveys we also provide a description of the previously undescribed subimago.

Methods

Distribution

The distribution of *I. abditus* was reviewed using published literature (Collier 1995; Hitchings 2001; Hitchings et al. 2015; Pohe et al. 2018a; Towns 1987; Towns & Peters 1979, 1996), a wide range of unpublished sources, and numerous stream surveys. Unpublished sources included records from the Auckland, Canterbury, Otago and New Zealand (Te Papa) Museums, New Zealand Arthropod Collection, specimens (confirmed by the lead author) from student theses (Arrieta 2010, 2012; Cook 2002; Pohe 2008), consultancy reports (Parkyn et al. 2006; Quinn & Halliday 1998; Quinn & Parkyn 1996), State of

the Environment monitoring data (1995–2011; MFE 2015), and personal communications with other freshwater ecologists (see Acknowledgements). For the stream surveys, imagos, subimagos and nymphs of *I. abditus* were obtained from 14 localities in 2013–2016 during an extensive nationwide light trapping and benthic sampling survey for mayflies and stoneflies (83 sites; see Winterbourn et al. 2017). Specimens were identified using standard New Zealand stream invertebrate texts (Towns & Peters 1996; Winterbourn et al. 2006). All records were plotted on a map of the North Island (Figure 6.1) and a complete catalogue of records is provided as supplementary material (Table S6.1).

Habitat preference

Habitat use by nymphs of *I. abditus* was investigated in 2015, in riffles, pools and backwaters of Mangere Stream in Northland. The study was undertaken during mid-September when nymphs of all sizes were still present in the stream (peak adult emergence is December–January). Mangere Stream was selected because *I. abditus* was known to be present there in moderately high numbers (Pohe et al. 2018a). Physico-chemical (environmental) variables were recorded at each sampling point before invertebrate sampling. A YSI Professional Plus handheld meter with Quatro multiparameter sensor was used to record pH, dissolved oxygen, conductivity and water temperature. Percentage cover of organic detritus (submerged leaves and twigs on the stream bed), estimated visually to the nearest 10%, was recorded for each sampling point. Depth of water at each sampling point was measured to the nearest centimetre, and mean stream velocity (three readings at each sampling point) was recorded with a Marsh–McBirney Flo-Mate (Model 2000). Sampling was conducted within an 8 hour period on a single day, within a 1.5 km study reach (centre 35.7106° S; 174.2452° E) that flowed through mixed podocarp–broadleaf forest and had stream widths of 1.9–4.2 m. Because no heavy rainfall events had occurred in the 2 months before sampling, backwater habitats had not been inundated by water from the main channel during that period.

Thirty quantitative benthic samples were taken in each habitat. Individual sampling positions for pools and riffles were selected at approximately 50 m intervals, whereas backwaters were sampled whenever they were encountered (backwaters were a less-common habitat). Riffles were sampled with a 25 cm × 25 cm Surber sampler (0.0625 m^2; 250 µm mesh), whereas both the pools and backwaters were sampled by disturbing the substratum within a 25 cm × 25 cm quadrat (0.0625 m^2) and scooping up dislodged material with a hand-net (300 µm mesh). Mayfly sampling was done systematically in an upstream direction to avoid the possibility of re-sampling individuals. The distinctive nymphs of *I. abditus* were counted in the field, their body lengths were measured to the nearest 1 mm, then most were returned alive to their respective sampling points. Small mayfly nymphs of uncertain identity were retained and checked in the laboratory by microscopic examination using a Nikon 10–63× stereomicroscope (Model SMZ800).

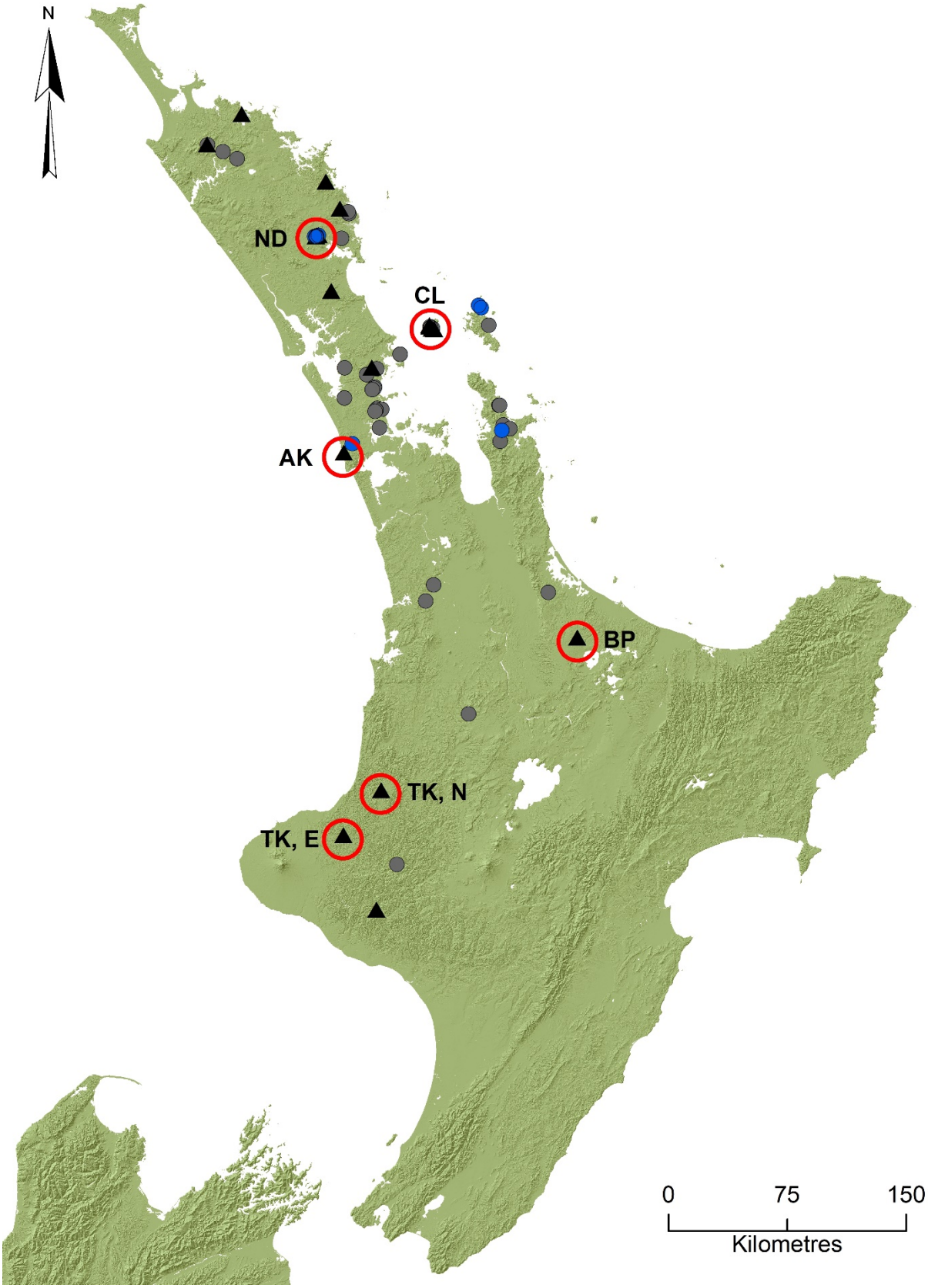

Figure 6.1 Distribution records for *Isothraulus abditus*. Blue points indicate locations of specimens recorded in published literature, grey points indicate locations of specimens recorded in unpublished literature (reports, theses, monitoring data, national specimen collections and personal communications) and black triangles indicate locations of specimens recorded in a national survey (Pohe 2013–2016). Red circles with corresponding codes indicate locations from which specimens for DNA analysis were obtained in this study. Code details are described in Table 6.1.

Specimens were assigned to one of three size classes, which also corresponded approximately to the following nymphal development stages: < 4 mm, wing pads absent; 4–8 mm, developing wing pads present but not extending over abdominal segment 1; and > 8 mm, wing pads extending beyond abdominal segment 1.

Table 6.1 Locations and site details used in the genetic structure study. Abbreviations in parentheses follow locality codes of Crosby et al. (1998) and correspond with locations in Figure 6.1.

Location (Code)	Site	Lat °S ; Long °E	Altitude (m)
Northland (ND)	Pukenui Forest	35.7046 ; 174.2616	210
Auckland (AK)	Piha	36.9388 ; 174.4671	15
Coromandel (CL)	Little Barrier Island	36.2114 ; 175.0527	35
Bay of Plenty (BP)	Rotorua	37.9604 ; 176.1675	365
Taranaki, North (TK, N)	Hutiwai Forest	38.8631 ; 174.7784	135
Taranaki, East (TK, E)	Pouiatoa Forest	39.1234 ; 174.5119	115

Genetic structure

DNA sequences were obtained from 123 specimens (imaginal and nymphal life stages) of *I. abditus*, sourced from six locations (Figure 6.1, Table 6.1), using the standard 'barcoding' fragment of the mitochondrial COI gene (Hebert et al. 2003). Sample locations were chosen that provided the largest number of *I. abditus* specimens available, and that also provided a broad geographic coverage of the known distribution. Only two individuals were obtained from a population in Auckland but were included in the data set because the samples were collected only 8 km from the type locality of *I. abditus*, and within the same forest. DNA was extracted from three legs of each specimen with an Extract-N-Amp Tissue PCR Kit (Sigma-Aldrich, St Louis, MO, USA), the legs being crushed and added to 25 µL Extraction Solution and 7.0 µL Tissue Preparation Solution, incubated in a thermocycler at 37 °C for 30–45 min, then at 95 °C for 3 min, after which 25 µL Neutralising Solution was added. The barcode fragment of the COI gene was amplified using the universal primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). Polymerase chain reaction (PCR) amplifications were performed in a final volume of 15 µL containing 2× Kapa2G Robust HotStart ReadyMix (7.5 µL), 0.35 µM of each primer (1 µL), nanopure water (5.5 µL) and 1.0 µL of template DNA (118–276 ng/µL). Thermal cycling parameters included an initial denaturation at 95 °C for 3 min, followed by 35 cycles at 95 °C for 15 s, 50 °C for 20 s, 72 °C for 30 s and a final 5 min extension at 72 °C. PCR products were purified with incubation in 2 µL ExoSAP-IT reagent and sequenced in both directions, using the same PCR primers used for amplification, by MacroGen (Seoul, South Korea). Forward and reverse sequences were aligned manually and trimmed to 658 base pairs (bp) using BioEdit Sequence Alignment Editor version 7.2.5 (Hall 1999) and deposited in GenBank (Benson et al. 2013) (Accession Numbers MH127930–MH127965).

Statistical analyses and phylogenetic inferences

To assess habitat preference, a *t*-test was used to compare differences in nymphal abundance between pools and backwaters (no nymphs were found in riffles). Differences in proportions of small, medium and large nymphs between the two habitats were compared with a chi-square test of association. Spearman rank correlation was used to determine whether within-habitat abundance of nymphs was associated with particular physicochemical variables.

Haplotype composition and a statistical parsimony network were calculated using TCS version 1.21 software (Clement et al. 2000). Evolutionary relationships were then displayed with a Median-Joining network (Bandelt et al. 1999) ($\varepsilon = 0$) in PopART version 1.7 (Leigh & Bryant 2015). Genetic distance within and among the six populations was estimated in MEGA 7.0 (Kumar et al. 2016) using the uncorrected *p*-distance method (see Collins et al. 2012), and also with the Kimura two-parameter model (Kimura 1980) to facilitate comparison with previously published studies. Our pairwise distance estimates employed the best-fitting nucleotide substitution model: a general time-reversible model with γ-distributed among-site rate variation and invariant sites (GTR + G + I; shape parameter 0.9566; bootstrap = 2000), which was calculated in MEGA 7.0 and selected using the corrected Akaike Information Criterion (Akaike 1973). Genetic diversity was estimated with Nei's (1987) gene (Hd) and nucleotide diversity (π) indices in Arlequin version 3.5.1.3 (Excoffier & Lischer 2010). Analysis of Molecular Variance (AMOVA; 2000 permutations) was also run in Arlequin to examine the effect of sample location on genetic variation using all data grouped as one 'New Zealand' data set, followed by the calculation of pairwise Φst population differentiation values (*F*-statistics). All results were considered to be statistically significant at $\alpha < 0.05$.

Results

Distribution

Isothraulus abditus has now been recorded from 55 streams in 37 localities (75 records in total: 9 published, 48 unpublished, 18 new). Its distribution is recorded from Mangonui Forest (35.0152° S) in northern Northland, to the vicinity of Taumatatahi (39.5432° S) near Whanganui (Figure 6.1), from an altitudinal range of 10–365 m above sea level. It has not been reported from the southeastern North Island, or the South Island, despite the considerable amount of sampling done there. Data from all previous sources (published and unpublished) indicated that of the 57 records, most (88%) were nymphal specimens, and only 12% contained adult material. However, in the recent nationwide survey, 166 individuals were collected from 14 localities, 92% of which were winged stages obtained by light trapping. Of the small number of nymphs collected, five were male and eight were female. Adult males were the most commonly trapped winged life stage (82%), followed by subadult males (10.5%), adult females (7%) and subadult females (0.5%). The largest light trap collections were taken beside first- and

second-order streams in mixed podocarp–broadleaf forests on Little Barrier Island (n = 46), in northern Taranaki (n = 35) and in Northland (n = 33), and of the 83 New Zealand streams sampled, *I. abditus* was only collected from forested sites.

Habitat preference

The three habitat types sampled (riffles, pools, backwaters) were distinctive, particularly with respect to water depth, water velocity and organic cover (Table 6.2). Riffles were shallow (mean 0.16 m), stony sections of stream bed with current velocities 0.3–0.9 ms^{-1}, whereas pools were 0.3–0.6 m deep, had current velocities < 0.1 ms^{-1} and contained deposits of organic matter. Backwaters were zones at the sides of the stream with negligible water movement and also contained organic deposits (pools and backwaters without organic matter were not sampled). Backwaters were either connected to the main channel by shallow transfer zones, or isolated from the main channel by gravel bars, but generally connected to it by subterranean flow.

Table 6.2 Mean water depth, water velocity, organic cover and abundance of nymphs of *Isothraulus abditus* in three habitats sampled in the Mangere River, Northland in September 2015. Values in parentheses are SEM.

	Depth (m)	Velocity (ms^{-1})	Organic cover (%)	Nymphs (per m^2)
Riffles	0.16 (0.005)	0.54 (0.028)	1 (0.5)	0
Pools	0.45 (0.014)	0.06 (0.004)	79 (3.0)	26.1 (7.5)
Backwaters	0.18 (0.010)	0.06 (0.003)	80 (3.9)	68.3 (12.1)

No *I. abditus* nymphs were found in riffle samples but 177 nymphs were taken from pools and backwaters. They were widely distributed within these two habitats, being present in 16 of 30 samples from pools and 24 of 30 samples from backwaters (means of 26/m^2 and 68/m^2, respectively; Table 6.2). Mean abundance of nymphs was significantly higher in backwaters than pools (t = 2.97, P < 0.01) but proportions of nymphs categorised as small, medium and large taken from pools and backwaters (n = 18, 22, 9 and 33, 61, 34, respectively) did not differ significantly (χ^2 = 2.51, P = 0.29). We also found that abundance of nymphs was strongly associated with organic cover at the individual quadrat level in both pools and backwaters (Table 6.3). Negative correlations with abundance of nymphs were found with dissolved oxygen and pH, which varied considerably at some backwater sampling points (dissolved oxygen 1.7–9.9 mg/L; pH 5.3–7.2; see Table S6.2 for physico-chemical data summary).

Table 6.3 Spearman rank correlations (r_s) between nymphal abundance of *Isothraulus abditus* and organic cover, dissolved oxygen and pH in pools and backwaters. Correlations with all other variables were non-significant ($P > 0.05$).

	Pools		Backwaters	
	r_s	*P*	r_s	*P*
Organic cover (%)	0.82	< 0.001	0.51	0.004
Dissolved oxygen (mg/L)	0.17	0.36	-0.54	0.002
pH	-0.10	0.58	-0.55	0.002

The most abundant macroinvertebrates found in backwater habitats (backwaters with and without *I. abditus*) were a second leptophlebiid mayfly *Arachnocolus phillipsi* Towns and Peters, 1979 and unidentified mites (Acari) and amphipods. The mayflies *Tepakia caligata* Towns and Peters, 1996 and *Zephlebia borealis* (Phillips, 1930), and small (< 30 mm total length) crayfish, *Paranephrops planifrons* White, 1842, were also recorded occasionally in backwater samples. Mites, various dipteran larvae and two mayflies, *Z. borealis* and *Zephlebia versicolor* (Eaton, 1899), were the most common invertebrates in pools, which were also inhabited by the mayflies *Zephlebia dentata* (Eaton, 1871) and *Neozephlebia scita* (Walker, 1853), the stonefly *Austroperla cyrene* (Newman, 1845), the caddisfly *Triplectides obsoletus* (McLachlan, 1862) and larvae of the dragonfly *Antipodochlora braueri* (Selys, 1871).

Genetic structure

Sequence data for the standard barcode fragment of the mitochondrial COI gene were obtained from 123 *I. abditus* specimens, collected at six locations across the North Island of New Zealand. There was no evidence of saturation in the data, mean nucleotide composition was T = 30.8%, C = 25.1%, A = 22.6% and G = 21.5%, and of the 658-bp positions, 39 were variable and 13 were parsimony-informative. Overall nucleotide diversity (π) was 0.004 and from the 123 sequences generated, 36 haplotypes were identified, 28 (78%) of which were singletons and 34 (94%) were unique to single populations. Mean *p*-distance among the 36 haplotypes was 0.7% (see Table S6.3 for individual haplotype *p*-distances and standard error estimates). Overall population haplotype diversity (Hd) was 0.7 (± 0.05 SD) and for the five populations represented by 12 or more individuals (i.e. excluding Auckland, $n = 2$) intra-population haplotype diversity ranged from 0.86 and 0.84 in eastern Taranaki and Northland, respectively, to 0.13 on Little Barrier Island (Table 6.4).

The haplotypes formed a single parsimony network (Figure 6.2), which comprised two star-shaped phylogenies and a poorly resolved cluster, all linked to a central common ancestor. One star-shaped phylogeny included 12 of the 15 observed Northland haplotypes (a Northland cluster), one of which was common to 11 individuals; the other 14 Northland haplotypes comprised only one to three individuals. *Isothraulus abditus* from Northland shared no haplotypes with individuals from other regions. However, three Northland haplotypes were included in the poorly resolved cluster, together with the two Auckland haplotypes (i.e. Northland was not monophyletic), and all three were more closely related to haplotypes

from regions further south (Figure 6.2). One common haplotype (*n* = 67) was shared among 74% of sequenced individuals from Rotorua, northern Taranaki, eastern Taranaki, and Little Barrier Island and formed the core of the second star phylogeny (a central North Island cluster). A second haplotype was shared among specimens from Little Barrier Island, northern Taranaki and eastern Taranaki, and was placed between the two star phylogenies and the poorly resolved cluster.

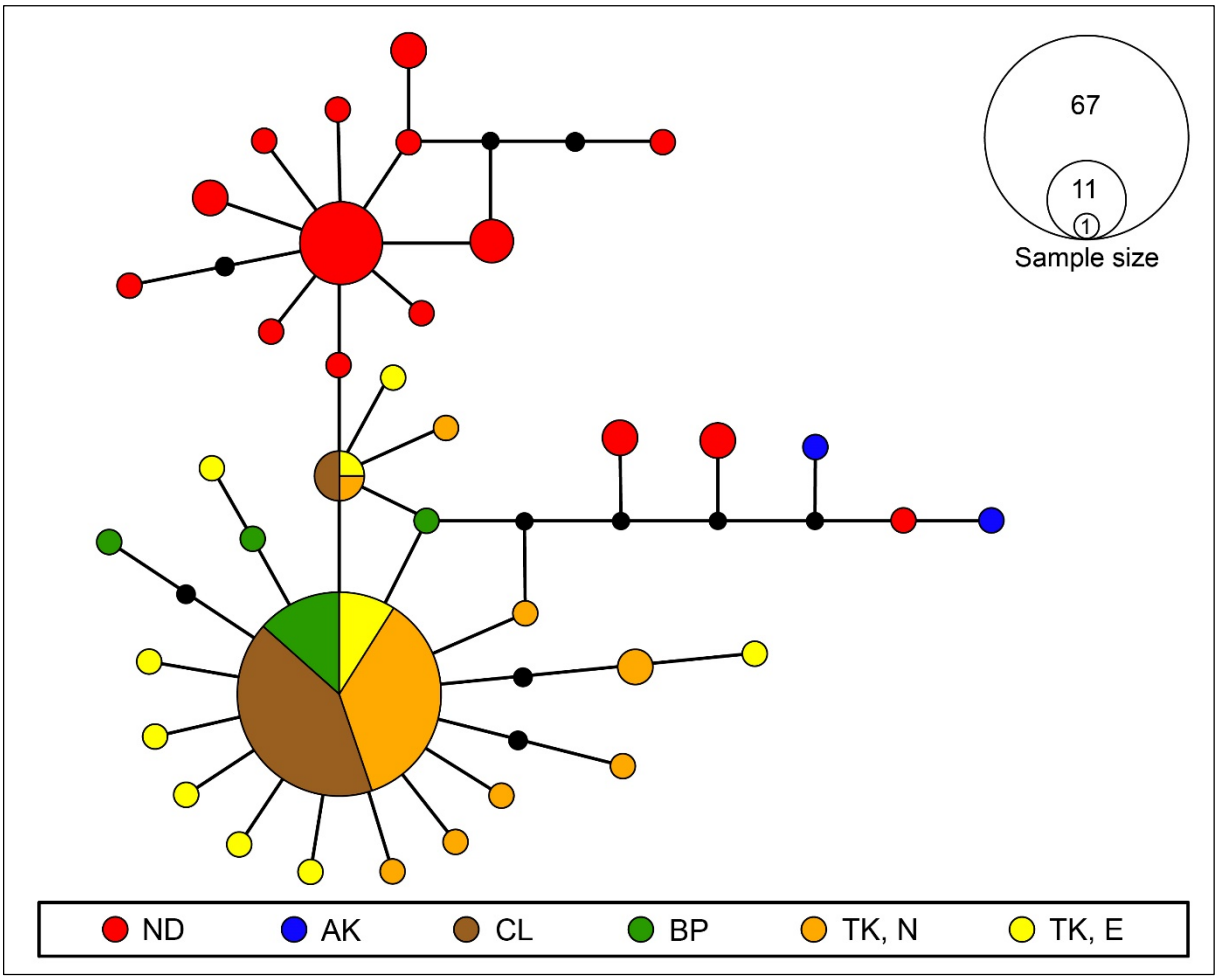

Figure 6.2 Median-Joining network for *Isothraulus abditus* based on a 658-bp fragment of the cytochrome *c* oxidase subunit I (COI) barcoding gene. Coloured points represent different sampling sites; the size of the points is relative to the frequency of the haplotypes. Black points indicate hypothetical haplotypes. Where haplotypes are shared by more than one location, relative haplotype frequency is indicated in the form of pie charts. Site codes: ND = Northland; AK = Auckland; CL = Coromandel (Little Barrier Island); BP = Bay of Plenty (Rotorua); TK, N = Taranaki, North; TK, E = Taranaki, East.

Table 6.4 Collection and genetic diversity information for *Isothraulus abditus* specimens used in the study, based on a 658-bp fragment of the cytochrome *c* oxidase subunit I (COI) barcoding gene. Abbreviations in parentheses follow locality codes of Crosby et al. (1998) and correspond with locations in Figure 6.1.

Location (Code)	*n*	Hap	Hd (± SD)	π (± SD)
Northland (ND)	31	14	0.84 (0.060)	0.0050 (0.0029)
Auckland (AK)	2	2	1.00 (0.500)	0.0046 (0.0053)
Coromandel (CL)	30	2	0.13 (0.079)	0.0002 (0.0003)
Bay of Plenty (BP)	12	4	0.46 (0.170)	0.0010 (0.0009)
Taranaki, North (TK, N)	33	9	0.48 (0.108)	0.0012 (0.0010)
Taranaki, East (TK, E)	15	10	0.86 (0.090)	0.0026 (0.0018)

n = number of individuals, Hap = number of haplotypes, Hd = haplotype diversity, π = nucleotide diversity.

Mean *p*-distance among groups ranged from 0.3% to 1.3% and within-groups from 0.2% to 0.7% (Table 6.5) suggesting populations belong to a single species. Maximum genetic *p*-distance was 1.8% between individuals from Auckland and Northland, and maximum within-group distances ranged from 0.2% to 1.7%. Analyses using the Kimura two-parameter statistic were identical, or very similar, to those obtained using *p*-distance so are not reported. AMOVA indicated that overall genetic differentiation in *I. abditus* was significant ($\Phi st = 0.2799$, $P < 0.001$); 28% of the variance was among populations and 72% within populations. In addition, significant mean pairwise differences were found between most populations (Table 6.6), with Northland and Auckland most strongly differentiated from other populations.

Table 6.5 Estimated evolutionary divergence of *Isothraulus abditus* based on a 658-bp fragment of the cytochrome *c* oxidase subunit I (COI) barcoding gene, presented as uncorrected *p*-distance (%). Between-group mean and maximum distances are presented above (grey shade text) and below the diagonal, respectively. Within-group mean distances are presented on the diagonal (bold values). Abbreviations in parentheses follow locality codes of Crosby et al. (1998) and correspond with locations in Figure 6.1.

Location (Code)	*n*	Hap	(ND)	(AK)	(CL)	(BP)	(TK, N)	(TK, E)
Northland (ND)	31	14	**0.7**	1.3	0.6	0.8	0.8	0.8
Auckland (AK)	2	2	1.8	**0.5**	1.1	1.1	1.1	1.2
Little Barrier Island (CL)	30	2	1.1	1.2	**0.2**	0.3	0.3	0.3
Rotorua (BP)	12	4	1.2	1.4	0.5	**0.3**	0.4	0.4
Taranaki, North (TK, N)	33	9	1.2	1.4	0.5	0.6	**0.4**	0.4
Taranaki, East (TK, E)	15	10	1.4	1.5	0.6	0.8	0.8	**0.4**

n = number of individuals, Hap = number of haplotypes.

Table 6.6 Pairwise *F*-statistics (Φst) for *Isothraulus abditus* based on corrected averages for the 658-bp fragment of the cytochrome *c* oxidase subunit I (COI) barcoding gene. Abbreviations in parentheses follow locality codes of Crosby et al. (1998) and correspond with locations in Figure 6.1. Note the Auckland population is only based on two individuals.

Location (Code)	(ND)	(AK)	(CL)	(BP)	(TK, N)
Northland (ND)	-				
Auckland (AK)	0.079	-			
Little Barrier Island (CL)	0.514***	0.436**	-		
Rotorua (BP)	0.351***	0.273	0.008	-	
Taranaki, North (TK, N)	0.341***	0.262	0.017*	-0.010	-
Taranaki, East (TK, E)	0.150***	0.071	0.129***	0.044	0.041*

Significant values are indicated as follows: *$P < 0.05$, **$P < 0.01$, *** $P < 0.001$.

Discussion

Before the present study, published distribution records for *I. abditus* were restricted to four localities (eight streams) in the Waitakere Ranges near Auckland, Great Barrier Island, Coromandel Peninsula, and in Northland. Our nationwide survey combined with existing unpublished records have extended the known distribution by adding 33 further localities (47 streams) in the Northland, Auckland, Coromandel, Waikato, Bay of Plenty, Taranaki and Whanganui regions. The known distribution of *I. abditus* now extends from Mangonui Forest in Northland, to the Waitotara Forest near Taumatatahi (Whanganui region), and is similar to those of other leptophlebiids such as *Arachnocolus phillipsi*, *Austronella planulata* (Towns, 1983), *Tepakia caligata*, *Zephlebia inconspicua* Towns, 1983, *Zephlebia nebulosa* Towns, 1996 and *Zephlebia tuberculata* Towns, 1996 (Towns & Peters 1996; S.R. Pohe, unpubl. data). Its distribution is also similar to the North Island distribution of the siphlaenigmatid mayfly *Siphlaenigma janae*, although *S. janae* is also found in the upper South Island (Pohe et al. 2018b). Although the distribution of *I. abditus* appears to be fragmented across the upper North Island, a hotspot for the species appears to be the Auckland region where its nymphs have frequently been recorded, especially by State of the Environment monitoring. One reason for this may be that topography in the Auckland region provides suitable habitat for *I. abditus* owing to its generally short catchments, rolling-hill landscape and forested first- and second-order streams. Further, because of the topography, Auckland's State of the Environment monitoring programme, by default, has a high proportion of monitoring sites within the habitat of *I. abditus*. In contrast, headwater streams are not sampled as often in other regions, which target their monitoring towards agricultural inputs and bathing sites lower in their catchments, supplemented by a small number of upper-catchment reference sites.

Our habitat study showed that nymphs of *I. abditus* occupied the slow-moving waters of pools and backwaters, and were absent from riffles, which have more rapid current velocities. This finding is in accordance with the comments of Towns and Peters (1979) and a later observation by Towns (1987) that they inhabited isolated pools connected by subterranean flow on Great Barrier Island. Towns (1987) also

speculated that they might be most common in intermittent streams. Because pools, backwaters and intermittent streams are less commonly sampled in general surveys than faster flowing riffles and streams with permanent flow, the specialised habitat of the nymphs of all sizes is likely to have contributed to the perception of their rarity. We also found strong positive correlations between nymphal abundance and organic cover in both pools and backwaters, indicating that organic matter strongly defines the habitat of *I. abditus*. However, whether it provides habitat *per se*, or a food supply, is not known, and requires investigation.

Backwaters were more variable habitats than pools and riffles in terms of temperature, dissolved oxygen, conductivity and pH, presumably reflecting variation in their degree of independence from the main channel. The significant negative correlations between nymphal abundance and dissolved oxygen and pH in backwaters were unexpected, but suggest that nymphs are tolerant of low oxygen concentration and fluctuating pH, at least in the short term. Nymphs of the leptophlebiids *Thraulus* and *Atalophlebia* have gill lamellae similar to those of *I. abditus*; their feather-like fringes increasing the surface area available to extract dissolved oxygen from the warm, still or slow-flowing waters of ponds, lakes and large rivers they inhabit (Gooderham & Tsyrlin 2002). The small body size of *I. abditus* nymphs and their distinctive feather-like gills may therefore aid survival in conditions with low oxygen concentrations, such as those sometimes found in backwater habitats. Interestingly, Tabacchi et al. (1993) found nymphs of *Thraulus bellus* Eaton, 1881 within the interstitial spaces of a temporary gravel floodplain pond in France, up to 0.8 m below the surface of the bed, and it is possible that as backwater pools dry, *I. abditus* could make use of the hyporheic zone as a refuge. A number of New Zealand stream invertebrates have been recorded within the interstitial spaces of the hyporheic zone (Adkins & Winterbourn 1999; Collier et al. 2004) and whether *I. abditus* makes use of this ecotone is worthy of further investigation.

Our genetic study indicated that *I. abditus* exhibited moderate haplotype diversity (Hd = 0.7), although mean *p*-distance was only 0.7%. The haplotype network comprised two main star-shaped clusters (phylogenies), one based on Northland and the other on the central North Island and Little Barrier Island. This separation suggests that populations in Northland may have been isolated and diversified within the region as proposed for two other mayflies, *Acanthophlebia cruentata* and *Siphlaenigma janae* (Pohe et al. 2018b; Smith & Collier 2001) and a stonefly, *Stenoperla prasina* (Newman, 1845) (Winterbourn et al. 2017). However, the processes shaping the evolutionary trajectory of Northland samples appears complex, with several of our Northland individuals forming a poorly resolved cluster together with Auckland individuals, and separate from the two main clusters. This may be a consequence of small sample size but could also be indicative of dispersal linking previously isolated populations. More targeted sampling within Auckland and Northland could highlight source–sink dynamics in these regions.

The second star phylogeny incorporated the three central North Island locations sampled, as well as Little Barrier Island. All of these locations shared a common haplotype comprising 55% of all 123 individuals sequenced, suggesting representation of a metapopulation. The presence in this network of individuals from Little Barrier Island suggests the island was likely colonised by dispersing individuals from central North Island rather than Northland, and the absence of unique haplotypes on Little Barrier Island supports the notion that dispersal may have been relatively recent. Little Barrier Island is the emergent part of a large dacite stratovolcano of mid-Pliocene to early Pleistocene age (Lindsay & Moore 1995) and has probably had repeated land connections and separations with Great Barrier Island, Coromandel Peninsula and the Northland mainland (Hamilton 1961). A phylogeographic study of the skink *Oligosoma smithi* (Gray, 1845) using the *ND2* mitochondrial gene indicated that individuals on islands of the Hauraki Gulf, including Little Barrier, formed a clade with skinks from the Coromandel Peninsula, whereas skinks from Northland formed a separate clade (Hare et al. 2008). A southern connection is also indicated by Hamilton's (1961, p. 15) statement that vegetation on Little Barrier Island 'resembles that of Coromandel Peninsula rather than that of the adjacent mainland at Cape Rodney [=Northland]'.

Isothraulus abditus was first noted as of potential conservation interest by Collier (1992, 1993) due to its known distribution at the time being restricted to two ecological regions (Auckland and Coromandel). Its conservation status was assessed by Molloy and Davis (1992, 1994) and classified as 'Category I', a grouping of species for which little information was known, but based on existing evidence, were considered threatened. It was subsequently relisted as 'Range Restricted $_{\text{Data Poor}}$' (Hitchmough 2002; Hitchmough et al. 2007) and most recently as 'At Risk (Declining A(2/1)) $_{\text{Data Poor, Range Restricted}}$' (Grainger et al. 2014). Based on new data we provide in this paper, and by applying the New Zealand Threat Classification System criteria (Townsend et al. 2008), *I. abditus* would be considered as 'Not Threatened'. However, it is difficult to classify aquatic invertebrate species using current threat classification criteria with confidence. First, difficulty arises because the 'population size' criterion for most aquatic invertebrate taxa is unknown, and difficult to estimate. Second, mature stages of some aquatic invertebrates, particularly insects, inhabit entirely different ecosystems, e.g. terrestrial riparian margins, from their juvenile stages, which are usually confined to aquatic habitats, e.g. groundwater, ponds and streams (and often to patches of suitable habitat within them) and hence the 'area of occupancy' criterion also falls short. Third, population trends are not known, and no capacity currently exists for population assessments to be conducted. As reported in the present study, the nymph of *I. abditus* has specific habitat requirements in forested streams and exists as geographically separated populations that show some genetic differentiation within the upper North Island. Consequently, some level of the 'At Risk' threat classification is appropriate.

The current fragmented distribution of *I. abditus*, especially in the northern half of its range, coincides with a part of New Zealand that was subject to intensive logging and forest degradation in the nineteenth century (Diamond 1966) and native forest tracts are now highly fragmented. Before logging it seems reasonable to speculate that *I. abditus* would have been much more widely distributed but due to its weak flying ability, present-day dispersal is likely limited. Effective conservation of the species requires that first- and second-order headwater tributaries be present within tracts of native forest and that they be given adequate protection from future development. National Parks and reserves largely serve this role at present and ideally will be supplemented by further protected areas that connect habitats, not only for the conservation of *I. abditus* but for freshwater faunas and ecosystems more generally. Furthermore, to conserve genetic variation within *I. abditus*, suitable forest habitat must be maintained throughout its distributional range, especially in Northland where genetic variability is greatest.

Description of male and female subimagos of *Isothraulus abditus*

Introduction

Towns and Peters (1979) described the male imago, female imago and nymph of *Isothraulus abditus* but not the subimago, which they stated was unknown, despite a female subimago being listed as a paratype. The adult and nymph were redescribed in their subsequent monograph on the New Zealand Leptophlebiidae (Towns & Peters 1996). We have now obtained male and female subimagos and take the opportunity to provide descriptions of them here. Descriptions and specimen dimensions are based on all available material: 23 male and four female subimagos. Body size ranges are provided with mean values in parentheses. Subimagos were confidently associated with adult material based on morphological characters, but were also associated by DNA analysis with nymphal and adult material. Collection localities are identified using the abbreviated codes of Crosby et al. (1998) and latitude and longitude coordinates use the New Zealand Geodetic Datum 2000 (version 20160701). All specimens are held in the personal collection of Stephen R. Pohe.

Description

***Isothraulus abditus* Towns and Peters, 1979**

Dimensions (mm). Male subimago: length of body 6.5–8.9 (7.6); forewings 7.5–9.8 (8.5). Female subimago: length of body 8.2–11.0 (9.5); forewings 9.1–12.1 (10.6).

Male subimago (in ethanol; see Figure S6.1A for image). Head grey-white with prominent conical ocelli; antennae filiform, shorter than width of head; eyes fused in midline, upper portion reddish-brown, lower portion black.

Thorax grey-white dorsally and ventrally; metanotum and posterior third of mesonotum each with a pair of grey longitudinal bands medially; mesopleura with a prominent 'bar' of dark pigment extending forward from a more diffuse patch of dark pigment on the posterior mesopleura and metapleura; metasternum with a transverse band of dark pigment. Wings translucent white; fringes of hairs on their posterior margins. Forewings with pale longitudinal veins; crossveins brown, those in cells C and Sc and near the middle of R1 surrounded by narrow clouds of dark pigment; diffuse patches of dark pigment at the fork of MA near the middle of the wing, and extending from the thickest crossveins in R1. Hindwings translucent; fringes of hairs on their posterior margins; venation pale; Sc about two-thirds length of wing. Legs grey-white; femora faintly yellowish.

Abdomen predominantly white dorsally and ventrally; ganglia not visible; terga 1–8 each with a narrow transverse dark band on hind margin; terga 8 and 9 dark grey, lighter in midline; tergum 10 and

external genitalia whitish; dark articulation points of nymphal gills prominent laterally on segments 1–7. Caudal filaments pale, as long as body.

Female subimago (in ethanol; see Figure S6.1B for image). As in male, except as follows. Head grey-white washed with black adjacent to eyes, around bases of ocelli and in the dorsal midline; eyes black, separated by approximately their own width. Abdomen darker than in male, yellowish-grey washed with brown-grey pigment medially giving the appearance of large paired pale patches laterally; terga 8 and 9 darker. Pairs of small yellowish maculae (spots) present anteriorly on terga 3 to 7; abdominal sterna paler than terga; ganglia not visible; sternum 7 with an egg guide reaching about the middle of sternum 8; sternum 9 rounded posteriorly with no terminal notch.

Material examined

ND. 1♂subimago, Tapapa Stm, Raetea Forest, Mangamuka, 35.1934 S; 173.4792 E, light trap, 10 January 2014, SR Pohe [POHE10120]. 1♂subimago, Punaruku Stm, Russell Forest, Punaruku, 35.3976 S; 174.3087 E, light trap, 16 December 2013, SR Pohe [POHE10844]. 3♂and 2♀subimagos, Kauri Stm [informal name], Pukenui Forest, Whangarei, 35.7046 S; 174.2616 E, light trap, 23–26 October 2013, SR Pohe [POHE00637, POHE01062, POHE01074, POHE01050, POHE01155]. 2♂and 1♀subimagos, Kauri Stm [informal name], Pukenui Forest, Whangarei, 35.7046 S; 174.2616 E, light trap, 22 December 2013, SR Pohe [POHE01746, POHE01784, POHE01783]. 3♂and 1♀subimagos, Pekapeka Stm [informal name], Pukenui Forest, Whangarei, 35.7141 S; 174.2508 E, light trap, 25–26 October 2013, SR Pohe [POHE01153, POHE01196, POHE01228, POHE01247]. 1♂subimago, Mangere Stm, Pukenui Forest, Whangarei, 35.7122 S; 174.2461 E, light trap, 23 October 2013, SR Pohe [POHE00719]. **AK.** 1♂subimago, Pohuehue Stm [informal name], Pohuehue Reserve nr Warkworth, 36.4535 S; 174.6515 E, light trap, 11 December 2013, SR Pohe [POHE03101]. **BP.** 2♂subimagos, Ohaupara Stm, Mangorewa Ecological Area, 37.9609 S; 176.1706 E, light trap, 01 February 2014, SR Pohe [POHE09931, POHE09934]. **TK.** 6♂ subimagos, Kotare Stm, Hutiwai Forest nr Kotare, 38.8631 S; 174.7784 E, light trap, 05 February 2014, SR Pohe [POHE12113, POHE12149, POHE12180, POHE12182, POHE12185, POHE12191]. 2♂subimagos, tributary of Waitara R., Pouiatoa Forest nr Purangi, 39.1234 S; 174.5119 E, light trap, 06 February 2014, SR Pohe [POHE06509, POHE06569]. **RI.** 1♂subimago, tributary of Waitotara R., Waitotara Forest nr Taumatatahi, 39.5432 S; 174.7624 E, light trap, 10 February 2014, SR Pohe [POHE03347].

Remarks. The subimago of *Isothraulus abditus* can be most easily distinguished from that of its closest relative *Tepakia caligata* by the characteristic forewing markings (as in the imago), the absence of dark brown tarsi, and the white rather than dark brown abdomen of the male. Although the abdomen of female *I. abditus* is darker than in the male it differs from that of *T. caligata* in having very prominent dark bands at the posterior margins of terga 1–8, prominent gill articulations on abdominal segments 1–7, and pairs of yellowish maculae anteriorly on terga 3–7. The egg guide is narrow and tube-like whereas

that of *T. caligata* is shorter and broader (Towns & Peters 1996). Note however that the egg guide of the subimagos we examined were shorter than that of the imago illustrated by Towns and Peters (1996). This may indicate that the egg guide lengthens at the final moult, since egg guides of adults collected at the same site extended to the posterior end of abdominal segment 8. Although colour patterns are useful for identification of both imagos and subimagos of *I. abditus* they are difficult to define precisely, and apparent colours are influenced by condition of preservation, microscope lighting and the nature of the background against which they are examined.

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Supplementary material

Table S6.1 Distribution records and metadata for *Isothraulus abditus* as at 12 January 2018. File prepared by Stephen R. Pohe, Pohe Environmental. Available for download (27 KB) at: https://www.tandfonline.com/doi/suppl/10.1080/03014223.2018.1464034/suppl_file/tnzz_a_1464034_sm9663.xlsx.

Table S6.2 Summary of physico-chemical data obtained in the habitat experiment on the Mangere Stream. CV = coefficient of variation (SD/Mean). Available for download (90 KB) at: https://www.tandfonline.com/doi/suppl/10.1080/03014223.2018.1464034/suppl_file/tnzz_a_1464034_sm9662.pdf.

Table S6.3 Individual haplotype *p*-distances (below the diagonal) and standard error estimates (above the diagonal in blue) for *Isothraulus abditus*, based on a 658-bp fragment of the COI barcoding gene. Available for download (25 KB) at: https://www.tandfonline.com/doi/suppl/10.1080/03014223.2018.1464034/suppl_file/tnzz_a_1464034_sm9661.xlsx.

Figure S6.1 A). Image of the subimago male of *Isothraulus abditus*. B). Image of the subimago female of *Isothraulus abditus*. Available for download (692 KB) at: https://www.tandfonline.com/doi/suppl/10.1080/03014223.2018.1464034/suppl_file/tnzz_a_1464034_sm9660.pdf.

Chapter 7 – *Synthesis*

"Science is expensive but if we're gonna do it, we should do it right."

Dr Steve O'Shea, 2004.

Me and my supervisory committee. **From left:** Jon Harding, Sharyn Goldstien, Steve Pohe and Mike Winterbourn.

Synthesis

The mayfly fauna of New Zealand are particularly interesting from a global perspective because all genera and species are endemic to the country, as are three families. The isolation of New Zealand from ancestral Gondwana during the Cretaceous period has created a unique fauna (Winterbourn 1980) which currently comprises 57 species in 20 genera and eight families (Hitchings & Hitchings 2018; Pohe 2018).

Our understanding of the New Zealand mayfly fauna has been based on work done by a relatively small number of researchers over a 165 year period, but the majority of studies have been since 1970. In his major taxonomic work on New Zealand Ephemeroptera Phillips (1931) recognised three families, Ephemeridae, Siphlonuridae and Leptophlebiidae. Subsequently, the burrowing mayfly genus *Ichthybotus* was moved from Ephemeridae to Ichthybotidae by Demoulin (1957), and in their systematic reviews of the family Siphlonuridae, McCafferty (1991) and Kluge et al. (1995) established the new families Ameletopsidae, Coloburiscidae, Nesameletidae, Oniscigastridae and Rallidentidae to contain some of the Southern Hemisphere fauna. Based on these changes, today's New Zealand fauna no longer have representatives of Ephemeridae or Siphlonuridae. The Leptophlebiidae has remained unchanged since the time of its creation, and comprises the largest number of New Zealand genera and species. Also, a new family, Siphlaenigmatidae, was erected by Penniket (1962) for a newly discovered New Zealand genus and species, *Siphlaenigma janae*.

Of the country's 57 species, more than half have been described in the last five decades. Seventeen were described by David Towns and William Peters in a series of publications and drawn together in their *Fauna of New Zealand* volume on New Zealand Leptophlebiidae (Towns & Peters 1996). More recently, Terry Hitchings and co-authors described 10 further species of *Deleatidium* (Leptophlebiidae), three species of *Nesameletus* (Nesameletidae) and a species of *Rallidens* (Rallidentidae) (see Chapter 2 for details). Winterbourn (2009) also erected a new genus (*Aupouriella*) for a newly described species of Leptophlebiidae from the far north of the country. In the present study I have recognised five tentative new species, but have not described them formally. They are two species of *Deleatidium*, two species of *Zephlebia* and one species of *Nesameletus*, and are largely based on various combinations of male genitalia, wing venation structure and patterning, abdominal colouration and nymphal gill structure. For the two species of *Zephlebia*, DNA barcoding (CO1 gene) has also been done (see Appendix 2) and I anticipate that genetic criteria will be valuable in determining their status and systematic positions. During this thesis the barcoding fragment of the mitochondrial COI gene was highly successful in discriminating between species of New Zealand mayflies (including species within genera). Sequencing of over 400 individuals with the COI barcoding gene, and targeted sequencing of the 28S gene (87 individuals) has been a valuable adjunct to my thesis research and has resulted in the creation of a DNA library that includes about 80% of the New Zealand mayfly fauna. This valuable resource is currently

lacking within New Zealand freshwater science realm, and I hope to develop it further as an open resource for freshwater scientists.

Interest in the affinities of the New Zealand Ephemeroptera has largely revolved around the Gondwanan connections shown by several families (e.g. Peters & Edmunds 1964; Edmunds 1972; Winterbourn 1980; Gibbs 2016). In his paper on New Zealand mayfly nymphs Phillips (1931, p. 401) quotes Tillyard (1926) in postulating the origin of New Zealand mayflies from a southern landmass incorporating "Tasmania, New Zealand, New Caledonia and even the Fiji Islands… and a connection with Antarctica". More recently, Towns and Peters (1996, p. 8) considered that all New Zealand mayflies have affinities with other fragments of Gondwana (Australia, South America, southern Africa, southern India, Sri Lanka, Madagascar and New Caledonia) and concluded that relationships of the New Zealand families are "consistent with ancient rather than modern contacts between land masses". They also indicated that the genera of New Zealand's largest mayfly family, Leptophlebiidae, belonged to six lineages with affinities to various Gondwanan elements. However, conclusions drawn from a molecular study based on two nuclear genes (*histone H3* and *28S ribosomal DNA*) by O'Donnell and Jockusch (2008) differ somewhat from those of Towns and Peters (1996) as seven of the eight New Zealand genera included in their study clustered together, and distinct from other members of their Palaeoaustral group. Their results therefore suggest that the New Zealand fauna may have been evolving independently of other Southern Hemisphere faunas for longer than previously hypothesised.

The distribution and abundance of mayflies has received considerable attention from ecologists in many parts of the world. The roles of abiotic and biotic factors in determining distribution patterns at various scales (continental, regional, local) have been investigated by many workers and useful reviews have been provided by Hynes (1970), Brittain (1982), (Brittain & Sartori 2003) and more recently by (Bauernfeind & Soldán 2012). Phillips (1931) noted that that little was known about the distribution of New Zealand mayflies prior to his contribution, which began to fill that gap. The extensive studies by William Peters and David Towns, and more recently Terry Hitchings, have been major advances in this respect, and have included the mapping of species distributions at the national level (see Towns & Peters 1996; Hitchings 2001; Hitchings & Staniczek 2003; Hitchings et al. 2015). My thesis had the documentation of species distributions, and the investigation of distribution patterns in relation to latitude and altitude, as a primary focus. To do this I undertook an extensive sampling programme throughout New Zealand at streams located in unmodified landscapes (mainly native forest) in order to enhance the prospect of obtaining "natural" mayfly assemblages that were less likely affected by land-use changes and anthropogenic pollutants. Sampling localities were distributed more or less evenly within latitudinal zones across the country and at a wide range of altitudes ranging from near sea level to 1000 m a.s.l. Two subsidiary surveys were also undertaken along an elevational gradient on Mount Taranaki from 120 to 1100 m a.s.l.

Light trapping was the principal sampling method chosen as it obtains imagos and subimagos that are more readily identified to species level (a necessary requirement of the study) than nymphs. It is well-known that mayflies are attracted to light, and light trapping had been used successfully to capture mayflies with powerful (160+ W) mercury vapour lamps at fixed sites in New Zealand (Norrie 1969; Ward et al. 1996). Many others have successfully used 6W or 8W fluorescent tubes for ecological studies and specimen collecting in New Zealand (see Pohe et al. 2018, and references within). Nevertheless, when I began my study it was unclear what wave lengths and light intensities were most useful for attracting mayflies (ultraviolet light being the favoured lights). I also needed my set-up to be portable for use in the field, often far from roads. Results of a preliminary study to resolve these questions are given in Chapter 3. Lights with blacklight-blue and blacklight spectra attracted most mayflies and caddisflies, and increasing the power intensity from 16 W to 32 W generally resulted in larger catches, although the increases were not statistically significant. Given my need for portability, and my desire to maximise catches, it was decided to use light traps comprising four 8-watt BLB fluorescent tubes (32 W) powered by two 12 V batteries, positioned horizontally above a white catching tray containing water and detergent. At each stream, three traps were placed on the stream bank, out of sight of each other (median distance of 140 m). As shown in Chapter 4 the lights captured large numbers of species and variable numbers of individuals of both sexes and life stages at each site. Light trapping was supplemented with benthic collections so that as many species as possible were found at each locality. Because light trapping so many sites required several months in consecutive years, and because the size of collections is influenced by many uncontrollable variables such as insect life cycles, weather, and moonlight, a conservative approach was taken to data analysis, emphasis being placed on presence-absence data (species richness).

New Zealand is well suited for undertaking a latitudinal study as it is a narrow country whose three main islands extend over almost 13 degrees of latitude. It also possesses considerable mountain ranges enabling altitudinal analyses. Few studies of mayflies appear to have explicitly investigated faunal composition and structure in relation to latitude of the kind undertaken here, in which sampling sites were standardised to as great a degree possible by locating them on small and moderate sized streams (1–20m wide; mean 5m) flowing through predominantly unmodified native landscapes. In contrast, numerous longitudinal studies of stream and river faunas have been made in various parts of the world, and although large rivers may extend over many degrees of latitude, such studies (e.g. Beketov 2008 and references within) differ fundamentally from mine. This is because downstream increases in river size are associated with changes in flow volume and velocity, as well as catchment and riverine variables such as the nature of riparian vegetation, substrate particle size, temperature, water chemistry and other factors that affect what animals, including mayflies, will live along the river continuum.

My results indicated that similar numbers of species inhabited the North and South islands, and even within most of the regional areas described by Crosby et al. (1998). That being said, species richness declined significantly at individual stream locations from north to south. Similarity of mayfly faunas found in 2-degree latitudinal bands also declined with distance such that those furthest north and south were most dissimilar. Fifteen species have now been found only on the North Island, 17 only on the South Island and 23 on both islands. The northern regions of the North Island, and especially Northland, are notable for the large number of species present, and for the presence of species restricted to them. These restricted species, in particular, are important drivers of the latitudinal gradient in species richness from north to south. Overall, my findings are consistent with Brittain's (1982) statement that in temperate regions of the world, the number of mayfly species generally decreases with increasing altitude, possibly because the higher temperatures found at lower altitudes enable additional species to grow and complete their life cycles.

In ectothermic animals including arthropods, body size is commonly correlated with temperature such that larger species occur in colder climates, and within species, size increases at higher latitudes and altitudes (e.g. Atkinson 1994). In their recent review of studies that have examined temperature–body size (T–S) relationships in insects, Horne et al. (2018) found that a latitudinal T–S relationship was commonly seen in species from 12 orders, although its nature could vary greatly among taxa, and could be obscured by other environmental variables. No mayflies were included in the review by Horne et al. (2018) but the one caddisfly species and five of six stonefly species showed increases in size as altitude increased. In the present study I found that both male and female *Coloburiscus humeralis*, one of New Zealand's most commonly occurring mayflies, increased in size with latitude and altitude in conformity with the T–S relationship. Thus, strong relationships were found with individuals becoming larger from north to south and from low to high elevations. My light trapping surveys at a smaller geographical scale on Mount Taranaki confirmed that *C. humeralis* grew larger at higher altitudes with a mean increase in wing length of 2.0 mm per 500 m rise in elevation. Furthermore, species richness declined markedly with altitude on the mountain with a pronounced decline above 730 m a.s.l. The nationwide latitudinal and altitudinal relationships shown by *C. humeralis* were stronger than those found for the stonefly *Stenoperla prasina* (based on material taken concurrently at lights in my nationwide survey) for which only males of *S. prasina* increased significantly in size with elevation on Mount Taranaki (Winterbourn et al. 2017).

The two final research chapters in this thesis were concerned with the distribution, ecology, genetics and conservation needs of two poorly known mayfly species. The first of these, *Siphlaenigma janae*, is the only representative of the endemic New Zealand family Siphlaenigmatidae, and the other, *Isothraulus abditus* was reported by Towns and Peters (1996) as one of New Zealand's rarest mayflies. My motivation to learn more about these species was driven by a perception that their future

conservation could only be ensured by a better understanding of their current distributions and habitat requirements. The studies of these two species also had a genetic component, their genetic structure being assessed with the barcoding fragment of the mitochondrial COI gene. Both species were found to have fragmented distributions restricted to small, forest streams. In the case of *S. janae*, these are typically low-gradient streams, which are increasingly being threatened by land-use change, notably at the type locality in Westland where the stream nymphs were first collected; the stream no longer exists as the land has been converted to pasture. My molecular analyses indicated the presence of geographically defined haplotype networks, suggesting limited dispersal and gene flow among populations, but insufficient genetic distance among them to realistically recognise separate geographic species. Overall, *S. janae* appears to be a species in decline and in need of serious conservation effort to protect its ever-disappearing forest stream environment.

Whereas *S. janae* is found in several parts of the North Island and in the northwest sector of the South Island, *I. abditus* has a distribution restricted to the northern half of the North Island only. A microdistribution study provided empirical evidence that its nymphs lived among detritus in pools and backwaters, as had been previously suggested (Towns & Peters 1996), but not the faster-flowing riffles. This slow-water habitat may have contributed to the limited number of historical records of the species as pools and backwaters tend not to be the sampling sites of choice of many workers. *Isothraulus abditus* exhibited moderate haplotype diversity, with Northland and Auckland populations most strongly differentiated from each other, and from all other populations. It has been proposed that populations of other mayfly species were isolated in Northland during the Pleistocene (e.g. Smith & Collier 2001) and have diversified there prior to recolonising streams further south. The genetic data obtained are consistent with such a scenario and add further weight to the importance of Northland as a refuge during periods of glaciation. Effective conservation of this species will require that low gradient, first- and second-order forest tributaries containing pools and backwaters are present within tracts of native forest and that they be given adequate protection from future development. Furthermore, to conserve genetic variation within *I. abditus* and *S. janae*, suitable forest habitat must be maintained throughout their distributional ranges, especially in Northland where genetic variability is greatest.

Finally, I was provided with an opportunity to investigate the invertebrate faunas of intermittent forest streams on Hauturu/Little Barrier Island, an important nature reserve in Auckland's Hauraki Gulf. A paper resulting from this investigation is included as Appendix 3. The survey was successful in adding 25 new aquatic invertebrate species records for the island, including seven mayfly species, and updated names of previously reported species last documented in 1964. No invertebrate species endemic to the island were found and faunal comparisons with literature accounts of adjacent mainland streams indicated that the island had assemblages of core taxa similar to them, although generally lower species richness. Seventeen species of mayfly have now been recorded from Hauturu, and includes species that

are either widespread within New Zealand, or present only in the northern half of the North Island. The most abundant nymphs were those of *Zephlebia borealis* and *Arachnocolus phillipsi*, both of which are common in headwater streams in the upper North Island.

In summary, I successfully accomplished the major objective of my research programme, which was to clarify and expand our knowledge of the New Zealand-wide distribution of our mayfly fauna. Nevertheless, there were a number of geographical gaps in my survey. I was only able to make collections at a limited number of localities on the Volcanic Plateau and East Coast regions in the central North Island, and in Fiordland and Stewart Island in southern New Zealand. Expanded sampling programmes in those areas would be well warranted. As my research involved a substantial programme of fieldwork throughout the country, and spanning three summer seasons, it also generated a substantial programme of laboratory work to identify the mayfly specimens (almost 30,000 all told). Without the outstanding taxonomic papers of David Towns and William Peters and the more recent contributions of Terry Hitchings the identification of much of my material would have been an impossible task, and even with them identification was not easy and involved many hours of microscope work to evaluate small differences in wing venation and external genitalia structure that define species. My research confirmed the notion that mayfly species richness is greatest in the north of the country but also showed that the diversity of species in the South Island is greater than had been anticipated. Nevertheless, at individual locations species richness declined with latitude from north to south and in line with studies elsewhere from low to high altitudes. The two studies on poorly known species with limited geographic distributions also highlighted the need for protection of their habitats not only for their future survival but also to maintain genetic diversity. Future research on New Zealand mayflies using the methods of molecular genetics will provide the opportunity to evaluate the true status of described and potential species, further our understanding of the genetic diversity of our mayfly fauna, and better understand its phylogenetic relationships.

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Appendix 1 – *Individual sampling site metadata*

Table A1.1 Zone 1 survey sampling locations, sites, and altitudes. * Denotes informal stream name.

Location and site	Waterway	Altitude (m)	Latitude; longitude (NZGD 2000)
Whangaroa 1	Sherman Stream	70	35° 01.1651′ S; 173° 43.0749′ E
Whangaroa 2	Waiarakau Stream	10	35° 00.8963′ S; 173° 43.0679′ E
Whangaroa 3	Waiarakau Stream	15	35° 00.9165′ S; 173° 42.9438′ E
Mangamuka 1	Tapapa Stream	75	35° 11.5611′ S; 173° 28.8019′ E
Mangamuka 2	Tapapa Stream	75	35° 11.6030′ S; 173° 28.7534′ E
Mangamuka 3	Tapapa Stream	80	35° 11.5778′ S; 173° 28.6795′ E
Puketi 1	Opaopao Stream	20	35° 16.6391′ S; 173° 41.2483′ E
Puketi 2	Waipapa River	30	35° 16.1645′ S; 173° 41.2845′ E
Puketi 3	Waipapa River tributary	40	35° 15.8347′ S; 173° 41.1373′ E
Russell 1	Punaruku Stream	30	35° 23.8673′ S; 174° 18.6682′ E
Russell 2	Punaruku Stream	30	35° 23.8581′ S; 174° 18.5201′ E
Russell 3	Punaruku Stream tributary	35	35° 23.7779′ S; 174° 18.5835′ E
Whananaki 1	Te Oriwa Stream tributary	40	35° 33.0212′ S; 174° 24.5810′ E
Whananaki 2	Te Oriwa Stream tributary	40	35° 33.0374′ S; 174° 24.5899′ E
Whananaki 3	Te Oriwa Stream tributary	40	35° 33.0507′ S; 174° 24.6054′ E
Waipoua 1	Waipoua River	75	35° 39.0282′ S; 173° 33.1284′ E
Waipoua 2	Waipoua River	75	35° 39.0891′ S; 173° 33.4105′ E
Waipoua 3	Waikohatu Stream	80	35° 39.0368′ S; 173° 33.4950′ E
Pukenui 1	Pukenui Stream*	200	35° 42.1500′ S; 174° 15.8190′ E
Pukenui 2	Pukenui Stream*	205	35° 42.2291′ S; 174° 15.7559′ E
Pukenui 3	Pukenui Stream*	210	35° 42.2746′ S; 174° 15.6976′ E
Mangere 1	Mangere Stream	120	35° 42.6247′ S; 174° 14.4987′ E
Mangere 2	Mangere Stream	125	35° 42.5921′ S; 174° 14.7197′ E
Mangere 3	Waterfall Creek*	130	35° 42.5449′ S; 174° 14.7282′ E
Mareretu 1	Finlaysons Brook	60	36° 01.2850′ S; 174° 21.5427′ E
Mareretu 2	Finlaysons Brook	65	36° 01.2924′ S; 174° 21.4576′ E
Mareretu 3	Finlaysons Brook	70	36° 01.2617′ S; 174° 21.4038′ E
Little Barrier 2[‡‡]	Tirikakawa Stream	30	36° 13.4161′ S; 175° 04.1343′ E
Little Barrier 4[‡‡]	Hut Bay Creek	15	36° 13.4915′ S; 175° 04.1496′ E
Little Barrier 5[‡‡]	Haowhenua Stream	40	36° 12.2879′ S; 175° 03.0356′ E
Pohuehue 1	Pohuehue Stream*	70	36° 27.1852′ S; 174° 39.0955′ E
Pohuehue 2	Pohuehue Stream*	75	36° 27.2112′ S; 174° 39.0914′ E
Pohuehue 3	Pohuehue Stream* tributary	80	36° 27.2304′ S; 174° 39.0737′ E

[‡‡] Five sites were sampled on Little Barrier Island, as part of the conjoint study reported in Appendix 3, but only three sites (2, 4 and 5), chosen for their spatial distribution and differing conditions, are considered in the thesis.

Table A1.2 Zone 2 survey sampling locations, sites and altitudes. * Denotes informal stream name.

Location and site	Waterway	Altitude (m)	Latitude; longitude (NZGD 2000)
Fantail Bay 1	Fantail Creek	20	36° 31.4407′ S; 175° 19.7724′ E
Fantail Bay 2	Fantail Creek	30	36° 31.3968′ S; 175° 19.8086′ E
Fantail Bay 3	Fantail Creek	40	36° 31.3723′ S; 175° 19.8708′ E
Cascades 1	Waitakere River	40	36° 53.1696′ S; 174° 31.1000′ E
Cascades 2	Waitakere River	50	36° 53.3037′ S; 174° 31.5646′ E
Cascades 3	Toetoeroa Stream	60	36° 53.4312′ S; 174° 31.8257′ E
Piha 1	Marawhara Stream	10	36° 56.4202′ S; 174° 28.0262′ E
Piha 2	Whakatai Stream	15	36° 56.3287′ S; 174° 28.0278′ E
Piha 3	Marawhara Stream	15	36° 56.4127′ S; 174° 28.1069′ E
Te Puru 1	Te Puru Stream	30	37° 02.7119′ S; 175° 32.0251′ E
Te Puru 2	Te Puru Stream	30	37° 02.7038′ S; 175° 32.0970′ E
Te Puru 3	Te Puru Stream tributary	30	37° 02.6978′ S; 175° 32.0232′ E
Hunua 1	Wairoa River tributary	130	37° 06.7275′ S; 175° 07.4709′ E
Hunua 2	Wairoa River tributary	110	37° 06.7598′ S; 175° 07.3584′ E
Hunua 3	Wairoa River tributary	130	37° 06.5447′ S; 175° 07.2315′ E
Port Waikato 1	Soroka Stream*	170	37° 21.8313′ S; 174° 47.6460′ E
Port Waikato 2	Soroka Stream*	160	37° 21.7370′ S; 174° 47.5144′ E
Port Waikato 3	Soroka Stream*	140	37° 21.6798′ S; 174° 47.3999′ E
Waitawheta 1	Waitawheta River tributary	120	37° 26.4893′ S; 175° 44.7207′ E
Waitawheta 2	Waitawheta River	90	37° 26.2391′ S; 175° 44.8099′ E
Waitawheta 3	Waitawheta River	90	37° 26.3321′ S; 175° 44.7374′ E
Te Tapui 1	Piakonui Stream tributary	150	37° 49.0271′ S; 175° 37.6974′ E
Te Tapui 2	Piakonui Stream tributary	160	37° 49.0975′ S; 175° 37.5711′ E
Te Tapui 3	Piakonui Stream tributary	170	37° 49.0808′ S; 175° 37.5194′ E
Pirongia 1	Kaniwhaniwha Stream	120	37° 56.2479′ S; 175° 04.4052′ E
Pirongia 2	Blue Bill Stream	125	37° 56.3339′ S; 175° 04.3380′ E
Pirongia 3	Blue Bill Stream tributary	130	37° 56.3713′ S; 175° 04.3684′ E
Rotorua 1	Ohaupara Stream	360	37° 57.6548′ S; 176° 10.2368′ E
Rotorua 2	Ohaupara Stream	365	37° 57.6258′ S; 176° 10.0492′ E
Rotorua 3	Ohaupara Stream	370	37° 57.6368′ S; 176° 09.9233′ E
Tokoroa 1	Okahukura Stream	545	38° 24.1379′ S; 175° 29.5192′ E
Tokoroa 2	Okahukura Stream tributary	550	38° 24.0541′ S; 175° 29.5218′ E
Tokoroa 3	Okahukura Stream	550	38° 24.0475′ S; 175° 29.5498′ E
Whareorino 1	Mangaotaki River tributary	320	38° 24.6887′ S; 174° 49.4355′ E
Whareorino 2	Mangaotaki River tributary	335	38° 24.7266′ S; 174° 49.3966′ E
Whareorino 3	Mangaotaki River tributary	335	38° 24.6898′ S; 174° 49.3599′ E

Table A1.3 Zone 3 survey sampling locations, sites and altitudes.

Location and site	Waterway	Altitude (m)	Latitude; longitude (NZGD 2000)
Pureora 1	Pangopangonui Stream	445	38° 38.3997′ S; 175° 29.8494′ E
Pureora 2	Pangopangonui Stream	450	38° 38.3754′ S; 175° 29.8755′ E
Pureora 3	Pangopangonui Stream	455	38° 38.3920′ S; 175° 29.9312′ E
Waikaremoana 1	Aniwaniwa River tributary	635	38° 44.5546′ S; 177° 10.3758′ E
Waikaremoana 2	Aniwaniwa River tributary	640	38° 44.6049′ S; 177° 10.3573′ E
Waikaremoana 3	Ruapapa Stream	600	38° 45.2745′ S; 177° 09.7221′ E
Gisborne 1	Te Arai Stream	190	38° 49.5695′ S; 177° 47.3600′ E
Gisborne 2	Te Arai Stream	200	38° 49.6920′ S; 177° 47.3758′ E
Gisborne 3	Te Arai Stream	220	38° 49.8144′ S; 177° 47.4096′ E
Kotare 1	Kotare Stream	130	38° 51.7413′ S; 174° 46.7040′ E
Kotare 2	Kotare Stream	135	38° 51.7884′ S; 174° 46.7045′ E
Kotare 3	Kotare Stream	140	38° 51.8280′ S; 174° 46.7317′ E
Pouiatoa 1	Waitara River tributary	110	39° 07.4051′ S; 174° 30.7685′ E
Pouiatoa 2	Waitara River tributary	115	39° 07.4058′ S; 174° 30.7123′ E
Pouiatoa 3	Waitara River tributary	120	39° 07.3997′ S; 174° 30.6379′ E
Taranaki 1	Waiongana Stream	595	39° 14.7151′ S; 174° 06.9418′ E
Taranaki 2	Waiongana Stream	600	39° 14.7353′ S; 174° 06.9185′ E
Taranaki 3	Waiongana Stream	605	39° 14.7456′ S; 174° 06.8610′ E
Timahanga Station 1	Mangataramea Stream	765	39° 24.3767′ S; 176° 14.6418′ E
Timahanga Station 2	Mangataramea Stream	770	39° 24.3677′ S; 176° 14.7111′ E
Timahanga Station 3	Mangataramea Stream	775	39° 24.3092′ S; 176° 14.6791′ E
Rotokohu 1	Waitotara River tributary	75	39° 32.5828′ S; 174° 45.7793′ E
Rotokohu 2	Waitotara River tributary	80	39° 32.5941′ S; 174° 45.7447′ E
Rotokohu 3	Waitotara River tributary	90	39° 32.6091′ S; 174° 45.6878′ E
Sentry Box 1	Sentry Box Stream	560	39° 40.0924′ S; 176° 17.0980′ E
Sentry Box 2	Sentry Box Stream	570	39° 40.1153′ S; 176° 17.0711′ E
Sentry Box 3	Sentry Box Stream	580	39° 40.1461′ S; 176° 17.0152′ E
Makohine 1	Makohine Stream	175	40° 13.4303′ S; 175° 48.6121′ E
Makohine 2	Makohine Stream	180	40° 13.4099′ S; 175° 48.6508′ E
Makohine 3	Makohine Stream	185	40° 13.4390′ S; 175° 48.6795′ E
Waewaepa 1	Makairo Stream	190	40° 25.2310′ S; 176° 01.6609′ E
Waewaepa 2	Makairo Stream	195	40° 25.2504′ S; 176° 01.6852′ E
Waewaepa 3	Makairo Stream	200	40° 25.2556′ S; 176° 01.7144′ E

Table A1.4 Zone 4 survey sampling locations, sites and altitudes. * Denotes informal stream name.

Location and site	Waterway	Altitude (m)	Latitude; longitude (NZGD 2000)
Collingwood 1	Kaituna River tributary	100	40° 42.6848′ S; 172° 34.1879′ E
Collingwood 2	Kaituna River	50	40° 42.7996′ S; 172° 34.1097′ E
Collingwood 3	Little Granity Creek	40	40° 43.0184′ S; 172° 34.8031′ E
Eketahuna 1	Makakahi River	360	40° 42.9056′ S; 175° 35.7335′ E
Eketahuna 2	Makakahi River	360	40° 42.9094′ S; 175° 35.6150′ E
Eketahuna 3	Makakahi River	360	40° 42.9102′ S; 175° 35.5078′ E
Totaranui 1	Totaranui Stream	25	40° 49.9072′ S; 172° 59.5951′ E
Totaranui 2	Totaranui Stream	30	40° 49.9634′ S; 172° 59.5574′ E
Totaranui 3	Totaranui Stream	35	40° 49.9601′ S; 172° 59.4812′ E
Carterton 1	Fern Stream (Waiohine R.)	160	40° 59.7199′ S; 175° 23.3429′ E
Carterton 2	Fern Stream (Waiohine R.)	160	40° 59.7354′ S; 175° 23.3534′ E
Carterton 3	Fern Stream (Waiohine R.)	165	40° 59.7188′ S; 175° 23.3978′ E
Harvey Bay 1	Harvey Bay Stream*	30	41° 07.3670′ S; 173° 44.3923′ E
Harvey Bay 2	Harvey Bay Stream*	40	41° 07.3880′ S; 173° 44.3139′ E
Harvey Bay 3	Harvey Bay Stream*	50	41° 07.4532′ S; 173° 44.2689′ E
Richmond 1	Elvy Stream (Pelorus R.)	35	41° 18.3083′ S; 173° 33.3312′ E
Richmond 2	Elvy Stream (Pelorus R.)	40	41° 18.3564′ S; 173° 33.3258′ E
Richmond 3	Elvy Stream (Pelorus R.)	45	41° 18.4336′ S; 173° 33.3544′ E
Robertson 1	Pukaka Stream	75	41° 22.9729′ S; 174° 00.9612′ E
Robertson 2	Pukaka Stream	75	41° 22.9245′ S; 174° 00.9999′ E
Robertson 3	Pukaka Stream	75	41° 22.8872′ S; 174° 00.9986′ E
Motueka 1	Conor Creek	340	41° 27.5588′ S; 172° 34.2700′ E
Motueka 2	Wangapeka River	280	41° 26.4891′ S; 172° 35.1002′ E
Motueka 3	Granity Creek	360	41° 28.3505′ S; 172° 34.2598′ E
Avon Valley 1	Five Mile Stream	460	41° 46.1435′ S; 173° 33.3767′ E
Avon Valley 2	Five Mile Stream	465	41° 46.1398′ S; 173° 33.3428′ E
Avon Valley 3	Five Mile Stream	470	41° 46.1449′ S; 173° 33.3046′ E
Lyell 1	Lyell Creek	90	41° 47.7663′ S; 172° 03.1053′ E
Lyell 2	Lyell Creek	95	41° 47.6840′ S; 172° 03.2163′ E
Lyell 3	Lyell Creek	100	41° 47.7083′ S; 172° 03.2730′ E
Rotoiti 1	Speargrass Creek tributary	860	41° 49.7450′ S; 172° 47.7665′ E
Rotoiti 2	Speargrass Creek	790	41° 49.9009′ S; 172° 47.2977′ E
Rotoiti 3	Speargrass Creek	800	41° 49.9807′ S; 172° 47.2028′ E
Reefton 1	Lankey Creek	230	42° 08.7247′ S; 171° 54.1033′ E
Reefton 2	Lankey Creek	235	42° 08.7327′ S; 171° 54.1482′ E
Reefton 3	Lankey Creek tributary	240	42° 08.6875′ S; 171° 54.2797′ E
Mororimu 1	Mororimu Stream	20	42° 12.9031′ S; 173° 51.9845′ E
Mororimu 2	Mororimu Stream	30	42° 12.8308′ S; 173° 51.9021′ E
Mororimu 3	Mororimu Stream	40	42° 12.8208′ S; 173° 51.8016′ E
Blackball 1	Moonlight Creek	175	42° 16.2831′ S; 171° 27.5871′ E
Blackball 2	Moonlight Creek	180	42° 16.2270′ S; 171° 27.4728′ E
Blackball 3	Moonlight Creek tributary	210	42° 16.1616′ S; 171° 27.4700′ E
Molesworth 1	(Fowler) Pass Stream	955	42° 18.8523′ S; 172° 45.5819′ E
Molesworth 2	(Fowler) Pass Stream	965	42° 18.8742′ S; 172° 45.4602′ E
Molesworth 3	(Fowler) Pass Stream	975	42° 18.8086′ S; 172° 45.3389′ E

Table A1.5 Zone 5 survey sampling locations, sites and altitudes. * Denotes informal stream name.

Location and site	Waterway	Altitude (m)	Latitude; longitude (NZGD 2000)
Lewis Pass 1	Garnet Stream A*	650	42° 31.3561′ S; 172° 24.4295′ E
Lewis Pass 2	Boyle River	620	42° 31.3006′ S; 172° 24.4520′ E
Lewis Pass 3	Garnet Stream B*	640	42° 31.3542′ S; 172° 24.4836′ E
Brunner 1	Eastern Hohonu River	195	42° 38.8477′ S; 171° 21.1849′ E
Brunner 2	Eastern Hohonu River	200	42° 38.9194′ S; 171° 21.2445′ E
Brunner 3	Eel Creek	190	42° 38.6055′ S; 171° 22.1135′ E
Hawkswood 1	Big Bush Gully Stream*	60	42° 39.9619′ S; 173° 25.2261′ E
Hawkswood 2	Big Bush Gully Stream*	65	42° 39.9854′ S; 173° 25.1757′ E
Hawkswood 3	Big Bush Gully Stream*	70	42° 39.9883′ S; 173° 25.1099′ E
Arthurs Pass 1	Bealey River tributary	940	42° 54.8617′ S; 171° 32.8567′ E
Arthurs Pass 2	Bealey River	980	42° 54.8332′ S; 171° 32.5729′ E
Arthurs Pass 3	Bealey River	1000	42° 54.8336′ S; 171° 32.4759′ E
Mt White Station 1	Peacock Stream*	550	42° 59.5931′ S; 171° 47.6565′ E
Mt White Station 2	Andrews Stream	550	42° 59.4097′ S; 171° 47.7874′ E
Mt White Station 3	Andrews Stream	555	42° 59.2975′ S; 171° 47.8514′ E
Harihari 1	Hercules Creek	115	43° 10.7463′ S; 170° 27.2997′ E
Harihari 2	Hercules Creek	125	43° 10.7817′ S; 170° 27.3123′ E
Harihari 3	Hercules Creek	130	43° 10.8081′ S; 170° 27.3319′ E
Oxford 1	Coopers Creek East Br.	370	43° 15.6343′ S; 172° 05.7080′ E
Oxford 2	Coopers Creek East Br.	375	43° 15.5790′ S; 172° 05.6777′ E
Oxford 3	Coopers Creek East Br.	380	43° 15.5266′ S; 172° 05.6763′ E
Porters Pass 1	Coach Stream	670	43° 18.4067′ S; 171° 45.3703′ E
Porters Pass 2	Coach Stream	680	43° 18.4082′ S; 171° 45.3082′ E
Porters Pass 3	Coach Stream	690	43° 18.3762′ S; 171° 45.2008′ E
Mt Somers 1	Bowyers Stream	455	43° 37.6430′ S; 171° 25.0520′ E
Mt Somers 2	Bowyers Stream	465	43° 37.6685′ S; 171° 24.9457′ E
Mt Somers 3	Bowyers Stream	470	43° 37.6763′ S; 171° 24.8838′ E
Paringa 1	Jamie Creek	25	43° 43.4105′ S; 169° 24.8577′ E
Paringa 2	Jamie Creek	35	43° 43.4982′ S; 169° 25.0372′ E
Paringa 3	Jamie Creek	45	43° 43.5714′ S; 169° 25.1490′ E
Okuti 1	Okuti River	90	43° 47.1089′ S; 172° 49.8788′ E
Okuti 2	Okuti River	100	43° 47.1350′ S; 172° 49.9705′ E
Okuti 3	Okuti River	120	43° 47.0752′ S; 172° 50.1473′ E
Haast 1	Harris Creek	40	43° 56.0465′ S; 169° 06.8739′ E
Haast 2	Harris Creek tributary	50	43° 56.0730′ S; 169° 06.8752′ E
Haast 3	Harris Creek	50	43° 56.0670′ S; 169° 06.8404′ E
Jackson Bay 1	Martyr River tributary	110	44° 07.4283′ S; 168° 33.2462′ E
Jackson Bay 2	Martyr River tributary	115	44° 07.4328′ S; 168° 33.3891′ E
Jackson Bay 3	Martyr River tributary	125	44° 07.4117′ S; 168° 33.4702′ E
Twizel 1	Gretas Stream	525	44° 14.8420′ S; 169° 52.6181′ E
Twizel 2	Gretas Stream	550	44° 14.9392′ S; 169° 52.9136′ E
Twizel 3	Gretas Stream	560	44° 14.9671′ S; 169° 52.9670′ E
Tekapo 1	Dalgety Stream	800	44° 19.7486′ S; 170° 35.1401′ E
Tekapo 2	Dalgety Stream	780	44° 19.9952′ S; 170° 35.2820′ E
Tekapo 3	Dalgety Stream	760	44° 20.2209′ S; 170° 35.3044′ E

Table A1.6 Zone 6 survey sampling locations, sites and altitudes. * Denotes informal stream name.

Location and site	Waterway	Altitude (m)	Latitude; longitude (NZGD 2000)
Rob Roy 1	Matukituki River West Br.	395	44° 30.2803′ S; 168° 43.3592′ E
Rob Roy 2	Rob Roy Stream	400	44° 30.1959′ S; 168° 43.2273′ E
Rob Roy 3	Rob Roy Stream	405	44° 30.1448′ S; 168° 43.2318′ E
Waimate 1	Hook River (Gunns Bush)	230	44° 39.7521′ S; 170° 58.5609′ E
Waimate 2	Hook River (Gunns Bush)	240	44° 39.8154′ S; 170° 58.4460′ E
Waimate 3	Hook River (Gunns Bush)	250	44° 39.8676′ S; 170° 58.3124′ E
Milford Sound 1	Tutoko River tributary 1	90	44° 40.3535′ S; 167° 58.0029′ E
Milford Sound 2	Tutoko River tributary 2	20	44° 40.7170′ S; 167° 57.9297′ E
Milford Sound 3	Cleddau River	15	44° 40.7711′ S; 167° 56.4556′ E
Lindis Pass 1	Lindis River	390	44° 41.7804′ S; 169° 29.6219′ E
Lindis Pass 2	Lindis River	380	44° 42.6090′ S; 169° 30.0649′ E
Lindis Pass 3	Lindis River	370	44° 43.1000′ S; 169° 30.2762′ E
Te Anau 1	Boyd Creek	310	45° 08.1730′ S; 167° 57.0052′ E
Te Anau 2	Boyd Creek	305	45° 08.0967′ S; 167° 57.0021′ E
Te Anau 3	Boyd Creek	295	45° 07.9638′ S; 167° 56.8932′ E
Oamaru 1	Waianakarua River Middle Br.	90	45° 16.3471′ S; 170° 43.8738′ E
Oamaru 2	Waianakarua River Middle Br.	90	45° 16.2830′ S; 170° 43.8824′ E
Oamaru 3	Waianakarua River Middle Br.	90	45° 16.2552′ S; 170° 43.7902′ E
Waikaia 1	Waikaia River	215	45° 33.2278′ S; 169° 01.2291′ E
Waikaia 2	Deer Creek*	220	45° 33.2882′ S; 169° 01.3670′ E
Waikaia 3	Post Office Creek	280	45° 32.7507′ S; 169° 02.5264′ E
Borland 1	Pig Creek	185	45° 46.5515′ S; 167° 31.8983′ E
Borland 2	Pig Creek	180	45° 46.4753′ S; 167° 32.0216′ E
Borland 3	Pig Creek	175	45° 46.4619′ S; 167° 32.2830′ E
Dunedin 1	Silver Stream	90	45° 48.4972′ S; 170° 25.3771′ E
Dunedin 2	Silver Stream	95	45° 48.3807′ S; 170° 25.4798′ E
Dunedin 3	Silver Stream tributary	100	45° 48.2864′ S; 170° 25.5112′ E
Black Gully 1	Black Gully Stream*	315	45° 53.6326′ S; 169° 21.1408′ E
Black Gully 2	Black Gully Stream*	310	45° 53.6486′ S; 169° 21.0832′ E
Black Gully 3	Black Gully Stream*	305	45° 53.6322′ S; 169° 21.0426′ E
Gore 1	Dunsdale Stream	105	46° 07.7772′ S; 168° 36.2096′ E
Gore 2	Dunsdale Stream	110	46° 07.6981′ S; 168° 36.2469′ E
Gore 3	Dunsdale Stream	115	46° 07.6415′ S; 168° 36.3470′ E
Otautau 1	Cascade Stream	80	46° 15.5774′ S; 167° 54.4743′ E
Otautau 2	Cascade Stream	75	46° 15.5796′ S; 167° 54.5597′ E
Otautau 3	Cascade Stream	75	46° 15.5820′ S; 167° 54.6257′ E
Invercargill 1	Ourawera Stream tributary	45	46° 19.8462′ S; 167° 50.0768′ E
Invercargill 2	Ourawera Stream tributary	55	46° 19.8074′ S; 167° 50.1147′ E
Invercargill 3	Ourawera Stream tributary	65	46° 19.7729′ S; 167° 50.1757′ E
Catlins 1	Catlins River	45	46° 27.3525′ S; 169° 28.3899′ E
Catlins 2	Catlins River tributary	55	46° 27.2279′ S; 169° 28.1862′ E
Catlins 3	Catlins River tributary	65	46° 27.2645′ S; 169° 28.1643′ E

Table A1.7 Zone 7 survey sampling locations, sites and altitudes. * Denotes informal stream name.

Location and site	Waterway	Altitude (m)	Latitude; longitude (NZGD 2000)
Bungaree 1	Golden Beach Stream*	10	46° 48.3743′ S; 168° 01.3879′ E
Bungaree 2	Golden Beach Stream*	15	46° 48.4013′ S; 168° 01.3878′ E
Bungaree 3	Golden Beach Stream*	15	46° 48.4106′ S; 168° 01.3649′ E
Oban 1	Mill Creek	35	46° 53.5758′ S; 168° 06.2229′ E
Oban 2	Mill Creek	40	46° 53.5762′ S; 168° 06.0188′ E
Oban 3	Mill Creek	45	46° 53.5054′ S; 168° 05.9426′ E
Rakeahua 1	Rakeahua Hut Creek*	5	46° 58.8968′ S; 167° 52.7201′ E
Rakeahua 2	SW Arm tributary	5	46° 58.5076′ S; 167° 54.0877′ E
Rakeahua 3	SW Arm tributary	10	46° 58.4732′ S; 167° 54.0910′ E

Appendix 2

Notes on undescribed 'species' included in Chapter 4

While outside the scope of my thesis research, I take the opportunity here to provide preliminary comments on observations of specimens that appear to differ from currently described species and are considered to be species in Chapter 4. I have made no attempt to describe or figure these taxa, but believe their inclusion in the thesis as tentative species, whether right or wrong, will benefit taxonomic advancement in the future, and importantly, will not impede it. The five "undescribed species" are given provisional tag-names based on patronymic association or regional localities. If descriptions do eventuate based on the data I present here, my preference would be they are given more traditional descriptive names.

In this appendix I also provide comments and opinions on several taxa suspected to include cryptic (hidden) species, some of which are supported by molecular analyses (sequenced to confirm their identity as there was some doubt relating to geographical or morphological differences). For details of molecular procedures and genetic distance calculations see 'Genetic identifications and the mayfly DNA library' in the Methods section of Chapter 4.

Deleatidium (Leptophlebiidae)

The genus *Deleatidium* is well known for the problems it creates for workers attempting to assign species-level identifications. Winterbourn (1977, p. 279) described the genus as "a taxonomist's nightmare", an opinion acknowledged by Towns and Peters (1996, p. 9) who also stated that "identification of described species [based on previous descriptions] has proved almost impossible". The monograph of Towns and Peters (1996) was a turning point in the methodical and detailed documentation of *Deleatidium* species, and included the reinstatement of two subgenera, *D.* (*Deleatidium*) and *D.* (*Penniketellum*)[††]. More recently, Hitchings (2008, 2009, 2010) and Hitchings and Hitchings (2016) have made further headway by describing additional *Deleatidium* species from the South Island. However, there is still work to be done, both on the described species, and on the numerous species still to be described. Therefore, while I have a high degree of confidence in the identification of many specimens (particularly male imagos), specimens of some species, or of some life stages, are rather nondescript, and we currently lack useful morphological characters to suitably define them. Accurate designation of specimens belonging to difficult taxa for which I had only female imagos, subimagos, or nymphs, was particularly difficult. To that end, I treated the identification of many difficult

[††] *Deleatidium* was previously divided by Phillips (1930) into *D*. (*Deleatidium*) and *D*. (*Atalophlebioides*), but on a different basis from that used to define the currently recognised subgenera. Ulmer (1938) subsequently raised *Atalophlebioides* to generic level, thus subgenera of *Deleatidium* became superfluous.

Deleatidium specimens with caution, and acknowledge that some identifications were at the level of morphospecies.

Deleatidium was divided by Winterbourn (1978) who recognised two informal species groups within the genus *Deleatidium*, the "*lillii* group" (abdominal gills with pointed apices) and the "*myzobranchia* group" (abdominal gills with rounded apices), to help differentiate the common nymphal forms. In some respects this is still the most useful demarcation for some groups within the *Deleatidium* (*D.*) subgenus, given our imprecise taxonomic knowledge, and given that some descriptions are difficult to interpret. Species designated as *D.* (*D.*) *myzobranchia* and *D.* (*D.*) *lillii* in my study are likely to be complexes of species, as they possess variations in colour, patterning, and morphological characters across New Zealand, particularly between North and South Island populations. A similar situation is likely for the widespread *D.* (*D.*) *autumnale* and *D.* (*D.*) *fumosum*.

In this thesis I recognise two additional *Deleatidium* species, *D.* (*D.*) "lyellensis" and *D.* (*D.*) "rakiura", both of which could be incipient species morphs of *D.* (*D.*) *lillii*. *Deleatidium* (*D.*) "lyellensis" was first figured and tentatively described by Moore (1968) but his descriptions were not of a high standard, and were never formally published. Specimens collected from the Lyell River (West Coast, South Island) during my nationwide survey have characters unlike any currently described species, and similar to those described by Moore (1968), although there are irregularities in some stated characters. After I had completed my laboratory work Hitchings and Hitchings (2016) published descriptions of two new species, *D.* (*D.*) *acerbum* and *D.* (*D.*) *kawatiri* and it is possible that my *D.* (*D.*) "lyellensis" is *D.* (*D.*) *kawatiri.* However further work is needed to confirm or discount this possibility. Interestingly, Hitchings and Hitchings (2016) other described species, *D.* (*D.*) *acerbum*, also resembles (in part) Moore's (1968) unpublished description of a further species, *D.* (*D.*) *pelori*. In both cases Hitchings and Hitchings (2016) designations would be the valid descriptions. The second species tentatively introduced in my thesis is *Deleatidium* (*D.*) "rakiura", collected during my nationwide survey from three locations on Stewart Island (Rakiura Island). It shows some similarities to both *D.* (*D.*) *lillii* and another recently described species, *D.* (*D.*) kiwa (Hitchings 2010) but appears to be distinct. The above mentioned species all warrant further taxonomic and molecular investigation, in all life stages.

Zephlebia (Leptophlebiidae)

The genus *Zephlebia* contains some of the earliest descriptions of New Zealand mayflies (Eaton 1871, 1899), which were initially placed in the genera *Leptophlebia* and *Atalophlebia*. Since that time *Atalophlebia* has had a complex taxonomic history and in 1961 Penniket transferred all the New Zealand species then in *Atalophlebia* to a newly created genus *Zephlebia*. Subsequently, some species attributed to *Zephlebia* were transferred to three newly established, monospecific genera, *Acanthophlebia*, *Austronella* and *Neozephlebia*. Comprehensive and masterfully figured reviews of *Zephlebia* and related genera were subsequently produced by Towns (1983) and Towns and Peters (1996), the latter including redescriptions of the five extant species known at the time, and descriptions of three additional species. The genus currently comprises eight well-defined species that exhibit a range of morphological variation across the species gradient. Although well-defined, members of some species are still difficult to identify in some life stages. A number of individuals collected during my nationwide survey differed from the currently described species, and thus I have included in this thesis two tentative *Zephlebia* 'species', and indicate two others of potential taxonomic interest.

The first tentative species, given the tag-name *Zephlebia* aff. *pirongia* sp. 1 (= *Zephlebia* cf. *pirongia* in Pohe et al. (2018)), was collected in the Pukenui Forest in Northland. It most closely resembles *Z. pirongia*, but differs in a number of morphological characters described by Towns and Peters (1996). In addition, preliminary DNA analyses of pairwise genetic distance comparisons of a 658-bp fragment of the COI gene suggest a mean interspecific distance of 14.1% (SEM 1.3%; 84 species pairs) from nine other *Zephlebia* congeners (Table A2.1).

Table A2.1 Mean pairwise genetic distances (COI gene; 658-bp) between *Zephlebia* aff. *pirongia* sp. 1 and its congener species. Described species (above the dotted line) are listed in order of pairwise distance. The number of species pairs and number of geographic locations that the data are derived from are also given.

Species	Pairwise distance (%)	SEM (%)	Species pairs	Geographic locations
Zephlebia borealis	10.5	1.1	6	3
Zephlebia pirongia	11.8	1.2	4	1
Zephlebia nebulosa	12.9	1.3	12	5
Zephlebia versicolor	14.6	1.3	16	8
Zephlebia tuberculata	15.1	1.3	10	2
Zephlebia dentata	16.1	1.3	12	6
Zephlebia spectabilis	16.2	1.4	6	3
Zephlebia inconspicua	Not assessed for genetic distance. No sequences available.			
Zephlebia "sp 1"	14.5	1.4	16	8
Zephlebia "rotokohu"	15.3	1.4	2	1

The second tentative 'species', given the tag-name *Zephlebia* "sp. 1" (=*Zephlebia* "hitchingsi" in my research notebook) was recorded across the upper South Island at ten survey sites. It is most similar to *Zephlebia dentata* and *Zephlebia nebulosa* in its nymphal form, and *Zephlebia pirongia* in its subimaginal and imarginal forms. Because of the similarities of different life-stages with other species it appears to have been previously attributed to a number of existing species. Preliminary DNA analyses of pairwise genetic distance comparisons indicate a mean interspecific distance of 16.6% (SEM 1.4%; 288 species pairs) from nine other *Zephlebia* congeners (Table A2.2).

Table A2.2 Mean pairwise genetic distances (COI gene; 658-bp) between *Zephlebia* sp. 1 and its congener species. Described species (above the dotted line) are listed in order of pairwise distance. The number of species pairs and number of geographic locations that the data are derived from are also given.

Species	Pairwise distance (%)	SEM (%)	Species pairs	Geographic locations
Zephlebia nebulosa	14.4	1.3	48	5
Zephlebia pirongia	15.0	1.4	16	1
Zephlebia versicolor	16.6	1.4	64	8
Zephlebia tuberculata	16.7	1.4	40	2
Zephlebia dentata	17.4	1.4	48	6
Zephlebia borealis	18.4	1.5	24	3
Zephlebia spectabilis	18.5	1.5	24	3
Zephlebia inconspicua	Not assessed for genetic distance. No sequences available.			
Zephlebia aff. *pirongia* sp. 1	14.5	1.4	16	1
Zephlebia "rotokohu"	17.6	1.4	8	1

Another species of potential interest is *Zephlebia nebulosa*. It is one of the more difficult *Zephlebia* species to identify, partly because it has been infrequently encountered (thus few specimens have been studied) and partly because it could be considered a 'cryptic species', having morphological characters similar or shared with other *Zephlebia* members, some of which are likely new species. During the nationwide survey, nine specimens were collected from seven geographical locations across the North Island. Morphological characters given by Towns and Peters (1996) identify all specimens as *Z. nebulosa* but DNA analyses of six specimens indicate northern and central North Island populations had a mean pairwise genetic distance of 10.4% (SEM 1.1%; 9 species pairs). The northern population, given the tag-name *Zephlebia* aff. *nebulosa* sp. 1, is likely a new species, but until more specimens can be located and assessed, the two groups are collectively reported in this thesis as *Z. nebulosa*.

Finally, a single subimaginal specimen collected from the Rotokohu Scenic Reserve north of Whanganui, had morphological characters found in several *Zephlebia* species (*Z. dentata*, *Z. tuberculata*, *Z. spectabilis*, *Z. inconspicua*, *Z. versicolor*). DNA analysis indicated a mean pairwise genetic distance from its closest congener, *Z. dentata*, of 11.0% (SEM 1.2%; 6 species pairs), and a mean of 15.6% (SEM 1.4%;

33 species pairs) with seven other described *Zephlebia* species (including *Z. dentata*). Therefore, it is likely to be an undescribed species and has been given the tag-name *Zephlebia* "rotokohu".

Nesameletus (Nesameletidae)

The Nesameletidae are an amphinotic family of 'swimming' mayflies of which New Zealand's representatives comprise five species in the genus *Nesameletus*. Early descriptions were published by Eaton (1883–1888) and Tillyard (1923), and more recently the genus was reviewed by Hitchings and Staniczek (2003) who provided redescriptions of the two extant species, and added three new species. While their monograph is expertly constructed, with clear descriptions that are supported by wonderful diagnostic figures, scanning electron microscope photography and taxonomic keys to all life stages, I seldom had confidence in making species designations, particularly for specimens that were not male imagos (the exception being *N. ornatus* (Figure A2.1) whose characters are most well-defined). Hitchings and Staniczek (2003) acknowledged that some species show geographic variation, and in their individual species descriptions all have dedicated subheadings for "Intraspecific variation" (except *N. murihiku*, which has a limited distribution). Hitchings and Staniczek (2003, p. 14) also stated that for the nymphal form "it may be necessary to confirm identification by rearing through to the imago". Clearly, more work is needed on identification characters to define the species. It seems likely that part of the identity confusion is due to the presence of a number of cryptic species that have yet to be acknowledged and described.

As a result of the taxonomic problems mentioned above, a number of molecular sequences were generated to aid identification. In addition, enough sequences were generated to enable a preliminary analysis of inter- and intra-specific genetic variation in *Nesameletus* (Table A2.3). This analysis could be improved by the addition of further sequences from specimens in other geographic locations. Preliminary DNA analyses of pairwise genetic distances indicate a mean interspecific distance of 15.7% (SEM 1.4%; 10 species pairs) across the five described species, with *N. flavitinctus* and *N. murihiku* most similar at 10.5%. However, considerable intraspecific genetic distance also exists within *N. ornatus*, *N. flavitinctus* and *N. austrinus*, and suggests additional cryptic species may be present.

Included in this thesis I suggest one additional tentative *Nesameletus* 'species', and indicate two others of potential interest. Specimens (40 nymphs, 2 adult females) collected from three sites in Fiordland appear to be morphologically unique, and have been given the tag-name *Nesameletus* "fiordlandi". No DNA sequencing has been done on these specimens yet, due to limited funds. Specimens (11 nymphs, 1 adult female) collected from below the Rob Roy Glacier in Mount Aspiring Nation Park were of potential interest. They were morphologically most similar to *N. austrinus*, with pairwise genetic distances indicating a mean distance of 8.5% (SEM 1.1%; 7.9–9.1%; 15 species pairs) from *N. austrinus*, thus have been recorded as *N.* "winterbourni" n. sp? in my research notebook. Specimens collected

during preliminary survey trials in November 2011 included *Nesameletus* nymphs from above Lake Tekapo (shallow braids of Godley River) that were difficult to identify and keyed out most closely to *N. vulcanus*, and are recorded as *N*. "tekapo" n. sp? in my research notebook. They may well be a new species. Finally, I would like to make a general comment about *N. ornatus* and *N. flavitinctus*, both of which show variation in morphology across their distributions. Pairwise genetic distances for *N. ornatus* indicate a mean separation between North and South Island populations of 7.2% (SEM 1.0%; 21 species pairs), and a lesser mean separation of 3.4% (SEM 0.7%; 12 species pairs) between Northland and the rest of the North Island. A similar situation is found in *N. flavitinctus*, but the largest mean genetic distances of 6.4% (SEM 0.9%; 9 species pairs) are between specimens from Northland (Pukenui Forest) and the central North Island (Mount Taranaki); North Island (Mount Taranaki) and South Island (Murchison) genetic distances averaged 4.1% (SEM 0.7%; 3 species pairs).

Figure A2.1 *Nesameletus ornatus* imago from Waipoua River, Northland.

Table A2.3 Specimen data, mean intraspecific (grey shade text) and interspecific pairwise genetic distances and pairwise ranges among sequences (COI gene; 658-bp) of described species of *Nesameletus* from New Zealand. Values in parentheses are SEM. *n* = number of specimens. GL = number of geographic locations.

Species class	*n*	GL	*N. ornatus*	*N. flavitinctus*	*N. murihiku*	*N. austrinus*	*N. vulcanus*
N. ornatus	8	8	4.7% (0.7%)[∞] 0.2–7.8% 28 species pairs				
N. flavitinctus	3	3	15.6% (1.4%) 14.1–16.9% 24 species pairs	5.6% (0.8%)[∞] 4.1–6.4% 3 species pairs			
N. murihiku	3	1	16.3% (1.4%) 15.5–17.0% 24 species pairs	10.5% (1.2%) 10.2–10.8% 9 species pairs	0.4% (0.2%) 0.2–0.6% 3 species pairs		
N. austrinus	8	3	16.1% (1.4%) 15.3–17.6% 64 species pairs	16.2 (1.5)% 14.1–17.2% 24 species pairs	17.2% (1.5%) 16.4–17.8% 24 species pairs	4.9% (0.7%)[∞] 0.2–9.1% 28 species pairs	
N. vulcanus	2	1	17.6% (1.5%) 17.2–18.5% 16 species pairs	15.5% (1.4%) 14.1–16.9% 6 species pairs	15.2% (1.4%) 15.0–15.5% 6 species pairs	16.5% (1.4%) 15.7–17.5% 16 species pairs	0.3% (0.2%) - 1 species pair

[∞] These values are high for intraspecific distances and are likely attributable to cryptic species whose placement by taxonomic keys currently falls within the described species. Note: Removal of outlier values, i.e. the potential new species, reduces the mean intraspecific pairwise distance to about 2.2%, a value commonly found within species.

Rallidens (Rallidentidae)

The family Rallidentidae is endemic to New Zealand, and is represented by just two species. *Rallidens mcfarlanei* was described in detail by Penniket (1966), based on specimens from Northland, though he included an addendum listing subsequent collection records from Northland, Auckland and Taumarunui in the North Island, and Kokiri and Pelorus Bridge in the South Island. *Rallidens platydontis* was described more recently by Staniczek and Hitchings (2014) from the South Island in which they included a number of comparative sketches and scanning electron microscope images of the two species in their publication. In my nationwide survey *Rallidens* material comprised 71 nymphal, 6 subimaginal (2 male, 4 female) and 33 adult specimens (all female), from ten locations distributed from Northland (upper North Island) to Oamaru (lower South Island). However, I have been unable to confidently assign species designations to any of these 110 individuals using current literature and macro-taxonomy (stereomicroscopy without the need for slide-mounted material). However, DNA barcoding of a 658-bp fragment of the COI gene was undertaken on specimens from eight locations (1–5 individuals from each): Northland (Waipoua), Auckland, Coromandel, inland Taranaki (near Purangi), Mount Taranaki, Ruahine Ranges (near Ashhurst), Collingwood and Oamaru. Exploratory results of pairwise genetic distance comparisons suggest three biogeographical groups of *Rallidens* (Figure A2.2), although it is acknowledged these were based on low numbers of specimens and thus no statistical analyses were undertaken. Group 1 comprised the Northland and Auckland specimens, Group 2 the central North Island specimens (including Coromandel) and Group 3 the South Island specimens. Analysis of pairwise genetic *p*-distances indicated Group 1 was 9.9% different from Group 2 and 9.7% different from Group 3, while Group 2 and Group 3 differed by 7.4%.

To contrast the above genetic distances with or New Zealand families, exploratory analysis of pairwise genetic *p*-distances undertaken on the New Zealand leptophlebiid genus *Zephlebia* (10 morphospecies; 842 species pairs) indicated a mean interspecific *p*-distance of 15.4% (SEM 1.3%) and a mean intraspecific *p*-distance of 1.3% (SEM 0.4%). Further, in the New Zealand genus *Oniscigaster* (Oniscigastridae), mean interspecific *p*-distance between the two acknowledged species (20 species pairs) was 8.1% (SEM 1.0%) and mean intraspecific *p*-distance was 1.5% (SEM 0.5%). While these findings are very much preliminary it suggests one of two scenarios for *Rallidens*; either it consists of a single widespread and genetically variable population of incipient species, or a third undescribed species (*Rallidens* "deathi" in my research notebook) exists in the central North Island (and possibly into the northwest of the South Island based on current Canterbury Museum records). A closer taxonomic inspection of material from the central North Island is warranted, and perhaps a taxonomic revision of specimens from all three biogeographical groups, complemented with a more detailed molecular study.

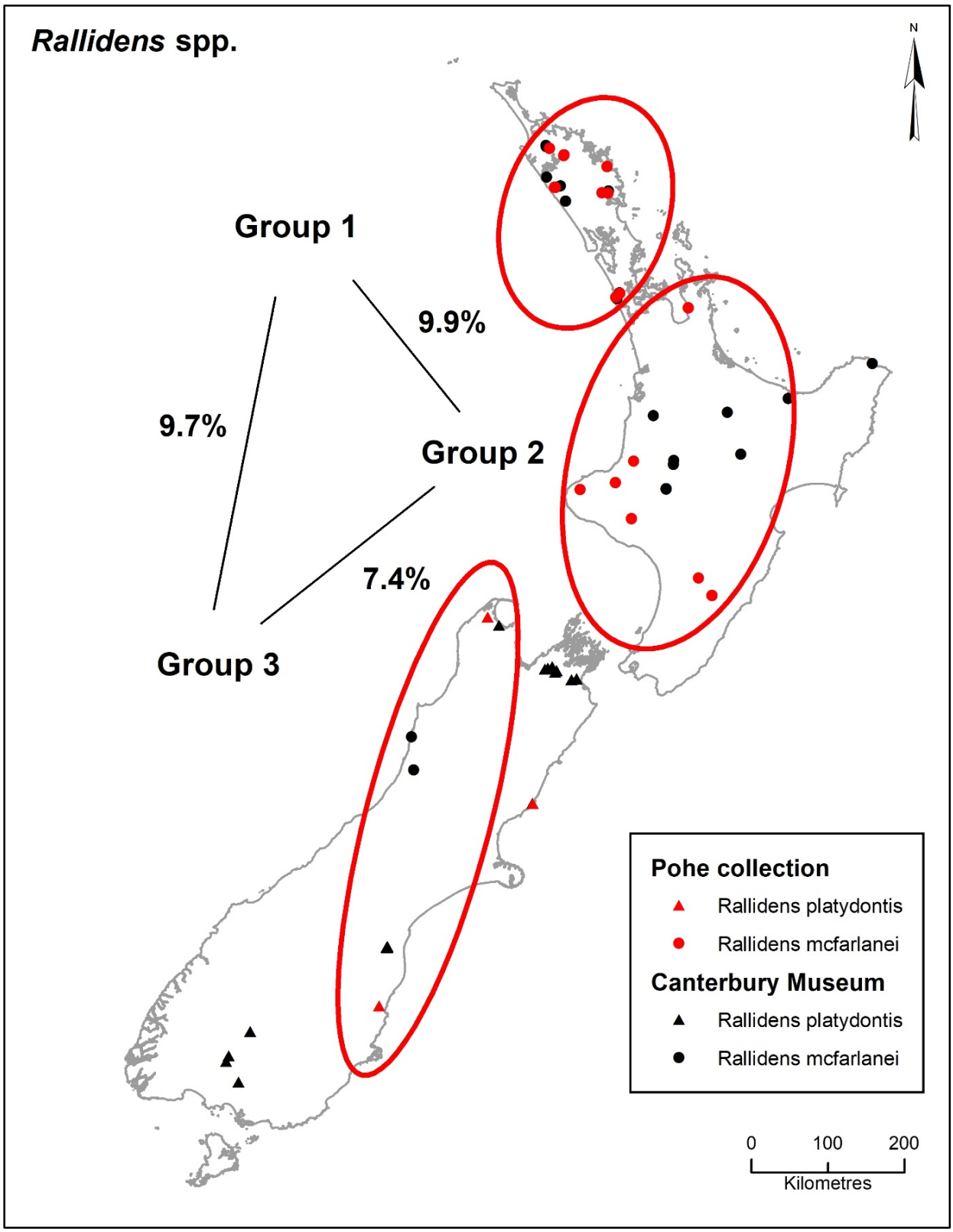

Figure A2.2 Three biogeographical groups of *Rallidens* as suggested by pairwise genetic distance comparisons of a 658-bp fragment of the COI gene.

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Appendix 3 – *Ephemeral stream invertebrate communities of Little Barrier Island*

Arial view of Hauturu-o-Toi/Little Barrier Island in northern New Zealand. DOC image library.

A3 preface

This manuscript describes a study of the invertebrate communities of ephemeral streams of the Little Barrier Island Nature Reserve in northern New Zealand and has been published as Stephen R. Pohe, M. Lyn Wade, Michael J. Winterbourn and Olivier J.-P. Ball. 2019. Invertebrate fauna of ephemeral streams on Hauturu-o-Toi/Little Barrier Island in northern New Zealand. New Zealand Journal of Zoology. https://doi.org/10.1080/03014223.2019.1576214. The journal's style and conventions have been retained but the format of tables and figures have been adjusted to fit the broader thesis layout. Supplementary material referenced in the text of this chapter is hyperlinked to the published online versions, and hyperlinks are also provided at the end of the manuscript.

Abstract

The invertebrate fauna of five ephemeral forest streams on Hauturu-o-Toi/Little Barrier Island in northern New Zealand was assessed in January 2014. Low summer flows restricted benthic sampling largely to pools that would, during periods of 'normal' flow, be main-channel riffle/run habitat. Additionally, adult stages of aquatic insects were sampled by light trapping. Fifty-three aquatic species/morphospecies were recorded during the study, including 25 new records, bringing total island species richness to 65. The fauna was dominated by Ephemeroptera and Trichoptera; species richness of Plecoptera and Diptera was low, and only single species of Mollusca and Crustacea were recorded. Species found were either common with broad New Zealand distributions, or species restricted to the North Island. No species was endemic to the island. Faunal comparisons with adjacent mainland streams indicated the island had similar assemblages of core taxa, but generally lower species richness, likely resulting from physiographic differences found there.

Keywords: Hauturu-o-Toi; Little Barrier Island; stream invertebrates; ephemeral streams; intermittent flow; New Zealand.

Introduction

Species richness on islands is frequently lower than in mainland regions because the small size of islands increases the likelihood of extinction and their isolation inhibits successful colonisation (Algar & Losos 2011). More limited resources and fewer habitats on islands may also reduce species richness relative to mainland areas (MacArthur & Wilson 1967). Habitat permanence is an additional factor affecting species richness and is particularly relevant for aquatic species. In general, streams of an ephemeral nature have lower species richness than equivalent perennial streams (Williams 1996), being highly variable environments comprising a dynamic mosaic of flowing water, disconnected pools and dry habitat patches (Datry et al. 2017). Pools typically expose organisms to higher and more variable water temperatures, higher nutrient concentrations and lower oxygen concentrations than flowing waters (Stubbington et al. 2017), making ephemeral streams potentially demanding environments for their inhabitants. Nevertheless, some invertebrate species are tolerant of such conditions (Richardson & Danehy 2007) and a number of studies have found that invertebrate communities of ephemeral streams comprise subsets of taxa found at perennially flowing sites (Boulton & Suter 1986; Storey & Quinn 2008; Stubbington et al. 2017 and references within). Most of the invertebrates in ephemeral streams are considered to be generalists with broad environmental preferences (Stubbington et al. 2017) although a few species are specialists found only where flow is intermittent (e.g. Towns 1985; Dieterich & Anderson 2000; Santos & Stevenson 2011).

Hauturu-o-Toi/Little Barrier Island (36.20° S; 175.08° E), hereafter called Hauturu, lies on the northern edge of the Hauraki Gulf, 75 km from central Auckland and 22 km from the nearest mainland. It is a roughly circular island of volcanic origin and is thought to have been formed by two separate events 3 million and 1.5 million years ago (Lindsay & Moore 1995). It has a land area of c. 3,000 hectares and is positioned in a warm humid climate with mild winters and an annual rainfall of c. 1400 mm (McEwen 1987). Mount Hauturu is the island's highest point at 722 m above sea level and most of the island's approximately 35 streams drop down steeply through precipitous ravines and are often ephemeral in nature (Hamilton 1961), particularly during summer.

In 1896 Hauturu became the first of New Zealand's Nature Reserves, having been purchased by the Crown in 1894 (Hamilton 1961). Although much of the island is now in a natural state, about one third of the vegetation had previously been felled or burnt (Veitch 2001). Following numerous eradication operations, present-day Hauturu is free of non-native browsing and predatory mammals and as a result is now home to an exceptional assemblage of native species including a high diversity of rare birds, reptiles and both species of New Zealand bat (O'Donnell et al. 2013; Hitchmough et al. 2016; Department of Conservation 2017; Robertson et al. 2017).

Although the terrestrial flora and fauna of Hauturu are well documented, few studies have been made on the freshwater fauna. Fish surveys carried out at eight streams on the island have collectively reported the presence of longfin eel (*Anguilla dieffenbachii*), banded kokupu (*Galaxias fasciatus*) and redfin bully (*Gobiomorphus huttoni*) and, although not a fish, the freshwater shrimp *Paratya curvirostris* (Wade 2014; NZFFD 2018). Wise (1956) first reported on the aquatic insects from streams in the south-western sector of Hauturu (sampled November 1954), presenting a list of 17 taxa belonging to seven orders. Subsequently, Winterbourn (1964) assessed the benthic invertebrate fauna of four streams in the same sector of the island (sampled August 1963), reporting an additional 22 taxa including the first records of freshwater snails, amphipods and flatworms. However, since that time juvenile and adult stages of many more species have been described from northern New Zealand, new genera have been established in several orders, and the fauna has undergone numerous nomenclatural changes (e.g. McLellan 1977, 1991, 1993; Cowley 1978; Towns & Peters 1996) resulting in many of the names used in the earlier surveys being no longer appropriate.

Given the close proximity of Hauturu to the mainland, stream invertebrate communities on the island are likely to have affinities with streams on the nearby mainland. However, due to the small size of Hauturu, and the intermittent nature of its streams, we hypothesised that stream invertebrate communities on Hauturu would differ in composition from those of perennially flowing streams on the mainland. The primary objective of the present study was to re-examine the macroinvertebrate fauna of the streams sampled in 1963 by Winterbourn (1964), particularly in the light of substantial advances in the taxonomy of stream invertebrates in the intervening half-century. We also investigated whether the stream invertebrate fauna of Hauturu had a distinct species composition by comparing it with previously

documented faunas of forested, stony, perennially flowing streams in three adjacent regions of the North Island mainland: the Coromandel Peninsula, Auckland and the vicinity of Whangarei.

Materials and methods

The streams

Most streams on Hauturu flow through fragmented volcanic rock, particularly on the northern and eastern coasts, and numerous waterfalls occur along their courses. During dry weather, particularly in summer, many of the streams have no surface water, although subsurface flow may be present (Hamilton 1961). On the southern and western sides of the island, streams broaden out in their lower courses and have partially alluvial beds. The streams enter the sea, either through boulder beaches, or via waterfalls, and the beds of most streams are dominated by boulders, cobbles and coarse gravels.

Five streams in the south-western sector of Hauturu were included in the present study (Figure 1, Table 1, Supplementary Table S1); four previously investigated by Winterbourn (1964) and Hut Bay Creek. At the sampling sites, four of the streams consisted of boulder–cobble dominated substratum and patches of gravels and/or bedrock. Substrata of one stream (Parihakoakoa Stream) consisted predominantly of bedrock, overlaid by a scattering of large cobbles, pebbles and gravels. At the time of sampling, stream channels had minimal surface flow (see Supplementary Figure S2) and water was limited in four of the streams to a series of pools connected by subsurface flow or occasionally, shallow (< 5 cm deep) sections of surface water. In contrast, Hut Bay Creek had shallow, continuously flowing surface water and may be spring-fed; a number of springs are known in that part of the island (Hamilton 1961; 2018 personal communication from Richard Walle, Hauturu Ranger). All streams flowed through native forest with overhead canopy cover (78–95%).

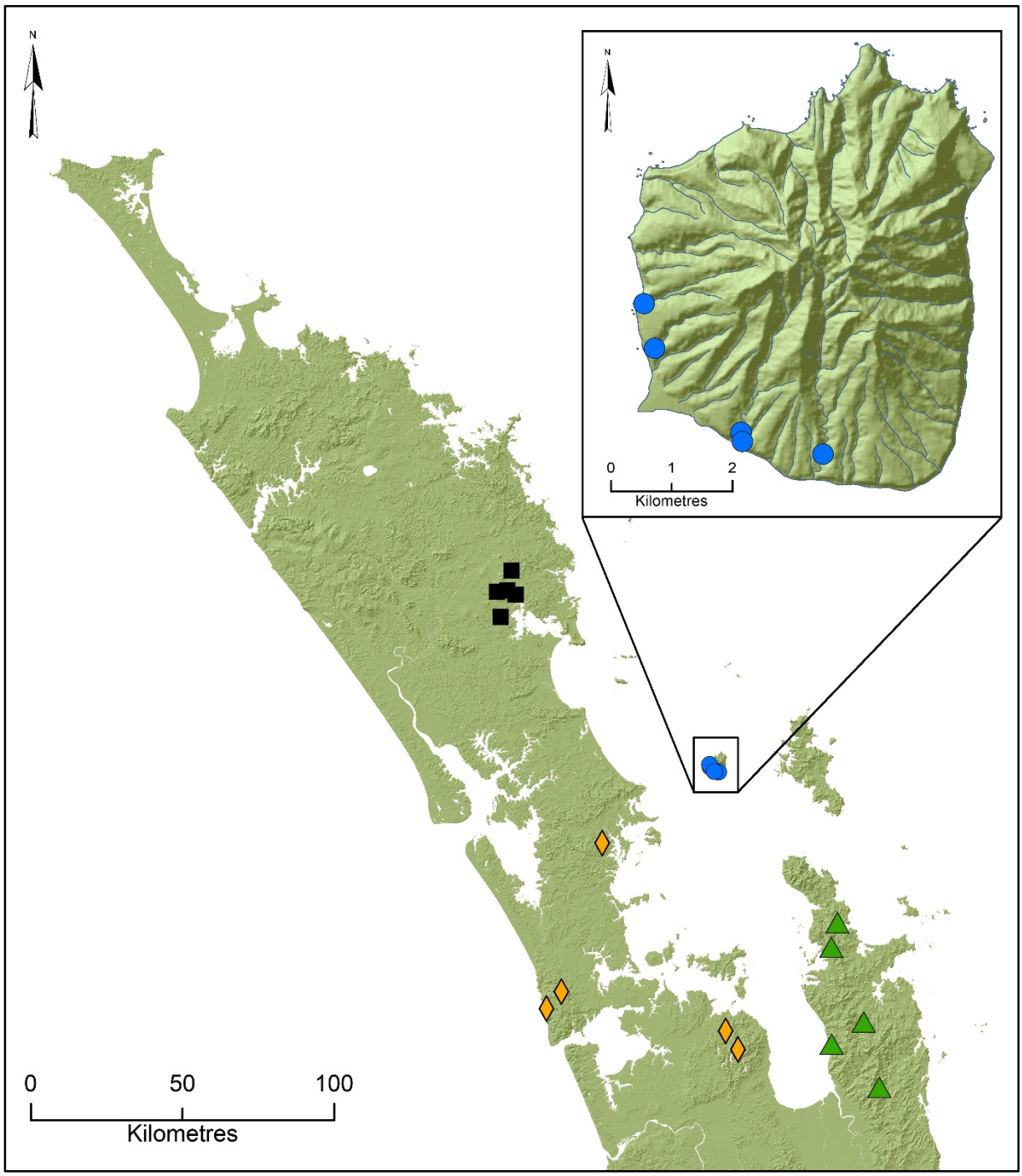

Figure 1. Map of northern New Zealand displaying locations of sampling sites used in the study. Enlargement indicates locations of sampling sites on Hauturu. For symbol details see Figure 3 and Supplementary Table S1.

Physico-chemical factors

Several environmental measurements were taken at each stream (Table 1). Water temperature (°C), pH, conductivity (µS cm^{-1}) and dissolved oxygen (g m^{-3}) were measured at three positions on each stream using a YSI Pro Plus meter at the time of invertebrate sampling. Wetted width of each stream channel was measured using a laser rangefinder (Bosch PLR-50) and overhead canopy cover was assessed with a convex spherical densiometer (Model-A, Forestry Suppliers Inc.) (Lemmon 1956, 1957). Hydrologic stability was assessed (by SRP) using the Pfankuch (1975) stability index, which incorporates 15 stability attributes of upper and lower banks, and the stream bed. Composition of the streambed substrate was

determined using a modified version of the pebble count method of Wolman (1954) and entailed measurement of the long axes of 50 particles (sizes ranging from fine gravels to boulders) collected at one pace intervals while walking upstream in a zig-zag manner. Air temperature was logged at each site using Hobo® pendant data loggers (Model UA-002-64, Onset Computer Corporation) to confirm that overnight air temperature was likely to have been suitable (> 12 °C) for aquatic insect flight activity (see Pohe et al. 2018 and references within).

Table 1. Physico-chemical measurements obtained for the five study streams in January 2014. Values given are means of the measurements made (see Methods). Temp. = water temperature, Cond. = conductivity, DO = dissolved oxygen, Width = wetted width of stream channel, Sub. = substrate size, Pfan. = Pfankuch stability index score.

Stream/Creek	Temp. (°C)	Cond. ($\mu S\ cm^{-1}$)	DO ($g\ m^{-3}$)	pH	Width (m)	Sub. (cm)	Pfan.
Parihakoakoa	15.4	114	0.8	6.6	1.80	12	79
Tirikakawa	18.3	99	2.1	5.8	1.95	38	53
Awaroa	16.6	75	5.3	6.2	2.77	23	59
Haowhenua	16.1	100	7.6	6.5	3.01	29	54
Hut Bay	16.7	126	8.3	7.0	2.81	29	56

Stream invertebrate sampling

Invertebrate sampling was carried out in the lower reaches of the five streams between 15 and 18 January, 2014. Each stream was sampled once. Samples were collected at three points on Parihakoakoa and Tirikakawa streams and at four points on Awaroa and Haowhenua streams and Hut Bay Creek (number of points sampled was based on available habitat but sampling effort was comparable at each stream). Because surface water was mainly confined to pools and occasional small riffles at the time of sampling, it was impractical to take strictly quantitative samples. Therefore, a D-net (width 33 cm; mesh size 0.25 mm) was swept through pools while benthic substrata and organic matter were being disturbed. Any large pieces of organic matter, e.g. tree branches, were washed in the D-net. Kick samples were taken from riffles where present. All samples were preserved in 70% ethanol.

Light trapping

Adult stages of aquatic insects were collected at one point per stream using a light trap comprising four 8-watt ultraviolet fluorescent tubes (total 32-watts; model F8T5 BLB tubes) placed over an 8-litre white plastic tray following Pohe et al. (2018). The tray was half-filled with water containing 30 ml of biodegradable detergent to reduce surface tension and so enhance retention of arriving insects. The trap was placed beside the stream and the lights activated for five hours from sunset. Light traps were operated once at each stream. Traps were emptied the following morning and Ephemeroptera, Plecoptera, Trichoptera and Megaloptera catches were removed from the trays in the field and preserved in 70% ethanol. Only representative selections of micro-caddisfly (Hydroptilidae) were

collected from Awaroa and Tirikakawa streams, due to the high numbers of individuals captured and the limited time available for processing them. The marine caddisfly *Philanisus plebeius* (Chathamiidae) was recorded in low numbers in light traps at three stream sites (Parihakoakoa, Awaroa and Haowhenua) but is not considered further as it does not have stream-dwelling larvae.

Identification of Hauturu invertebrates

Stream invertebrates and light-trapped insects from Hauturu were examined under a dissecting microscope at 10–63x magnification and were identified to the lowest taxonomic level possible using Marshall (1975), Ordish (1984), Neboiss (1986), Ward (1995), Towns and Peters (1996), Winterbourn et al. (2006) and Chapman et al. (2011). In most cases (c. 80%), individuals were identified to species level. For the remainder, there was a high level of confidence for discrete morphospecies designations and therefore, hereafter the term species is used for all Hauturu 2014 sampling results. Identifications of adult caddisflies were checked and corrected where necessary by trichopteran specialist, Brian Smith (Freshwater Biologist, NIWA Hamilton).

Faunal comparison with adjacent mainland regions

Hauturu stream invertebrate faunas were compared with faunas previously reported from streams in three mainland regions (Coromandel Peninsula, Auckland and Whangarei) in close proximity to the island (Figure 1). The mainland data had been collected by Regional Councils using the standard 'hard-bottomed' stream sampling protocol (Stark et al. 2001) for State of the Environment (SoE) reporting (MFE 2015) and were obtained for two dates for each of five streams within each mainland region (see Supplementary Table S1 for details). The Hauturu dataset comprised the data collected by Winterbourn (1964) from four streams, and the data obtained in the present study (five streams), and therefore provided for a near-balanced comparison with respect to numbers of streams and times sampled. For our comparisons, coded abundance data (0, 1, 5, 20, 100; see Stark 1998) were generated for each stream, based on averaged results of each pair of sampling events. This was more complex for the Hauturu stream pairs, due to taxonomic additions and changes introduced since the 1963 sampling was undertaken. Best effort was made to match historical species lists with currently recognised taxa, an approach for which we have a good level of confidence in the outcome. All streams used in the comparison (mainland and Hauturu) were located in native forest with closed canopies, as indicated by Google Earth Pro Version 7 and NZ Topo Map (http://www.topomap.co.nz) software, and had predominantly stony substrata as indicated for the mainland sites by use of the 'hard-bottomed' stream sampling protocol. The mainland stream sites were widely distributed within each region. Because mainland data had been identified to the taxonomic level used to calculate macroinvertebrate community indices (MCI) (Stark 1985, 1998) for SoE reporting, Hauturu data were adjusted to that level for regional comparisons. Thus, most of the 102 taxa included in the Hauturu–mainland comparison were identified to genus or family, rather than species.

Statistical analyses

A cluster analysis based on presence/absence data for all species (benthic and light-trapped individuals combined) was used to compare the faunal composition of the five streams on Hauturu. The Bray–Curtis similarity index and unweighted paired-group average linkage (UPGMA) were used for clustering in the software package Paleontological Statistics (PAST) version 3.20 (Hammer et al. 2001). Taxonomic richness in the four regions (n = 20, five streams in each region) was compared with a Kruskal–Wallis H-test, followed by Mann–Whitney U-tests to find where significant differences lay using PAST 3.20. Nonmetric multidimensional scaling (NMS) incorporating the Bray–Curtis distance measure was used to ordinate invertebrate communities in taxonomic space for stream sites on Hauturu and the three mainland regions (n = 20, five streams in each region). Cube-root transformed coded abundance data were calculated for each stream so that an element of relative abundance was included in the ordination. The solution with the lowest stress in two dimensions was selected as only small reductions in stress were obtained when additional axes were included. Monte Carlo simulations (250 runs) were used to test the statistical significance of the ordination. Nonmetric multidimensional scaling in conjunction with the Bray–Curtis distance measure is a robust method for analysing community data sets, which often have a high proportion of zeros, leading to non-normally distributed data (McCune & Grace 2002). Spearman rank correlation was used to test whether there was an association between taxonomic richness and ordination scores for axes 1 and 2. The multi-response permutation procedure (MRPP) with the Bray–Curtis distance measure was used to test whether macroinvertebrate stream communities from the four different regions were significantly different. Indicator species analysis (ISA) was used to identify which invertebrate taxa were most influential in determining regional differences (Monte Carlo test with 1000 runs). NMS, MRPP and ISA were all run in the statistical software PC-ORD version 6.22 (McCune & Mefford 2011).

Results

Physico-chemical factors

Canopy cover of the five streams on Hauturu ranged from 78 to 95%, mean wetted widths from 1.8 to 3.0 m and mean substrate size (maximum particle length) from 12 to 38 cm (Table 1). Hydrologic stability, as measured by the Pfankuch index, was assessed as ‘good’ or ‘fair’ in all five streams, which also had similar water temperatures (15.0–19.5 °C). pH of four streams was slightly acidic (5.8–6.8), whereas Hut Bay Creek was circumneutral (6.9–7.1). Dissolved oxygen (DO) of stream/pool water varied markedly amongst sampling sites with Parihakoakoa (0.7–1.0 g m^{-3}) and Tirikakawa (1.9–2.3 g m^{-3}) water having particularly low oxygen concentrations. In contrast, DO exceeded 4.8 g m^{-3} in the other three streams (mean 7.1 g m^{-3}) and in Hut Bay Creek, mean saturation of 8.3 g m^{-3} was recorded.

Hauturu aquatic invertebrate fauna

Fifty-three invertebrate species were identified from the five streams on Hauturu in 2014. They included aquatic stages collected from the stream benthos, and adult stages of insects taken at lights (Table 2). Numbers of species found at individual streams ranged from 17 to 32 (Table 3). Forty-seven (89%) of the 53 species were insects, of which three mayfly (Ephemeroptera) species and 10 caddisfly (Trichoptera) species were only collected at lights. Gastropoda, Amphipoda, Tricladida and Nematomorpha were represented by single species and only two oligochaete species were found. Trichoptera and Ephemeroptera were the best represented insect orders with 18 and 14 species, respectively. The most abundant benthic species collected in the five streams were the mayflies *Zephlebia borealis* and *Arachnocolus phillipsi*, the caddisfly *Polyplectropus altera,* the snail *Potamopyrgus antipodarum* and larval Chironominae (Diptera).

Of the 110 adult and subadult mayflies captured in light traps at the five streams *Isothraulus abditus, Z. borealis* and *Acanthophlebia cruentata* were taken in greatest numbers. No species was present in light traps at all five streams but two (*A. cruentata* and *Z. borealis*) were trapped at four of them. *Stenoperla prasina* was the only adult stonefly taken at lights (3 streams). Ten freshwater caddisfly species belonging to eight families were collected by light trapping, but only *Polyplectropus altera* was taken at all five streams. The dobsonfly *Archichauliodes diversus* was taken at three streams. Cluster analysis of presence–absence invertebrate data showed that the faunas of Tirikakawa and Awaroa streams were most similar (74%; Figure 2), and that the fauna of Parihakoakoa Stream, which had the fewest species, was most divergent.

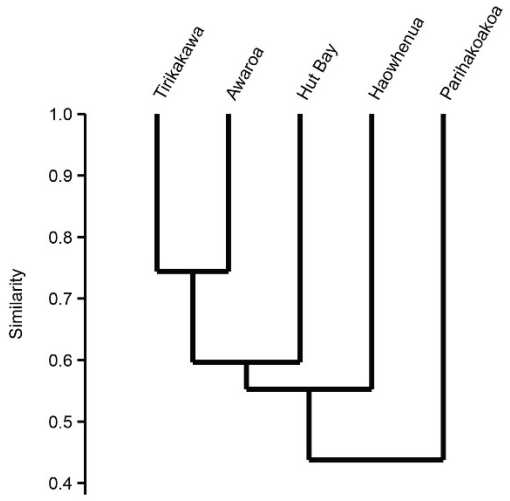

Figure 2. Cluster of five Hauturu streams based on presence/absence of all benthic and light-trapped invertebrate species collected, using the Bray–Curtis similarity index and UPGMA linkage.

Table 2. Macroinvertebrate fauna recorded from five streams on Hauturu, 15–18 January 2014. L = adult insects collected by light trapping, S = stream dwelling taxa collected by kick/sweep sampling.

			Parihakoakoa		Tirikakawa		Awaroa		Haowhenua		Hut Bay	
			L	S	L	S	L	S	L	S	L	S
Ephemeroptera	Ameletopsidae	*Ameletopsis perscitus*			✓	✓		✓		✓	✓	
	Coloburiscidae	*Coloburiscus humeralis*							✓			
	Ichthybotidae	*Ichthybotus hudsoni*			✓				✓		✓	
	Leptophlebiidae	*Acanthophlebia cruentata*			✓	✓	✓	✓	✓	✓	✓	✓
		Arachnocolus phillipsi		✓		✓		✓		✓	✓	✓
		Deleatidium (D.) fumosum			✓	✓	✓	✓				
		Deleatidium (D.) cf. *lillii*								✓		
		Isothraulus abditus	✓	✓	✓	✓		✓		✓	✓	✓
		Mauiulus cf. *aquilus*			✓							
		Neozephlebia scita										✓
		Zephlebia borealis			✓	✓	✓	✓	✓	✓	✓	✓
		Zephlebia dentata									✓	✓
		Zephlebia inconspicua				✓					✓	✓
		Zephlebia spectabilis								✓		
Plecoptera	Austroperlidae	*Austroperla cyrene*				✓				✓		
	Eustheniidae	*Stenoperla prasina*			✓	✓	✓	✓		✓	✓	✓
Trichoptera*	Conoesucidae	*Olinga feredayi*	✓		✓	✓			✓	✓	✓	✓
		Pycnocentria funerea								✓		
	Helicopsychidae	*Helicopsyche zelandica*			✓	✓	✓	✓		✓	✓	✓
	Hydrobiosidae	*Hydrobiosis gollanis*	✓								✓	
		Hydrobiosis parumbripennis			✓							
		Hydrochorema crassicaudatum							✓			
		Psilochorema mimicum	✓		✓		✓				✓	
		Tiphobiosis cf. *cowiei* (♀)							✓			
		Tiphobiosis kleinpastei									✓	
	Hydropsychidae	*Hydropsyche fimbriata*							✓			
	Hydroptilidae	*Oxyethira albiceps*			✓	✓	✓					
	Leptoceridae	*Triplectides obsoletus*							✓			

			Parihakoakoa		Tirikakawa		Awaroa		Haowhenua		Hut Bay	
			L	S	L	S	L	S	L	S	L	S
	Oeconesidae	*Oeconesus maori*									✓	✓
		Pseudoeconesus sp.								✓		
		Tarapsyche olis	✓									
	Philopotamidae	*Hydrobiosella mixta*			✓		✓		✓			
	Polycentropodidae	*Plectrocnemia* sp.		✓						✓		
		Polyplectropus altera	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Megaloptera	Corydalidae	*Archichauliodes diversus*			✓		✓	✓	✓			✓
Hemiptera	Veliidae	*Microvelia macgregori*		✓						✓		✓
Coleoptera	Hydraenidae	*Homalaena* sp.								✓		
	Hydrophilidae	*Paracymus* sp.										✓
	Scirtidae	Sp. A (hooded type[†])		✓								
		Sp. B (narrow type[‡])		✓		✓						
Diptera	Chironomidae	*Ablabesmyia mala*				✓				✓		✓
		Orthocladiinae sp.								✓		
		Polypedilum sp.		✓		✓		✓		✓		✓
	Culicidae	*Culex pervigilans*		✓		✓		✓				
	Dixidae	*Paradixa harrisi*		✓				✓		✓		✓
	Sciomyzidae									✓		
	Tipulidae	Eriopterini sp.		✓								
Amphipoda	Paracalliopiidae	*Paracalliope fluviatilis*		✓								✓
Gastropoda	Tateidae	*Potamopyrgus antipodarum*		✓		✓		✓		✓		✓
Nematomorpha	Gordiidae	*Gordionus maori*						✓				
Oligochaeta	Lumbriculidae	*Lumbriculus variegatus*								✓		✓
	Tubificidae	*Limnodrilus udekemianus*						✓				
Tricladida	Dugesiidae	*Neppia* sp.										✓

[*] The marine caddisfly *Philanisus plebeius* (Chathamiidae) was also collected in light traps at three streams (see Methods).

[†] as in Figure 183 of Winterbourn et al. (2006)

[‡] as in Figure 182 of Winterbourn et al. (2006)

Table 3. Numbers of taxa collected from Hauturu during the 2014 survey from benthic samples, numbers of EPTM (Ephemeroptera, Plecoptera, Trichoptera, Megaloptera) species collected in light traps and total numbers of taxa recorded at each stream.

Stream/Creek	Benthic taxa	EPTM at lights	Total taxa
Parihakoakoa	13	6	17
Tirikakawa	18	16	24
Awaroa	16	10	19
Haowhenua	24	12	32
Hut Bay	22	16	27
All of Hauturu	40	28	53

Faunal comparison with adjacent mainland regions

One hundred and two MCI level invertebrate taxa were recorded in the dataset for the four-region comparison. Of these, 46 (45%) were found on Hauturu, 52 (51%) on the Coromandel Peninsula, 81 (79%) in Auckland and 80 (78%) near Whangarei (Table 4). Nine of the 18 mayfly taxa, five of eight stoneflies, 13 of 24 caddisflies and eight of 20 dipterans recorded in the four regions were found on Hauturu. However, only one of the seven freshwater snails (*Potamopyrgus antipodarum*) and one of eight crustaceans (*Paracalliope fluviatilis*) were found on Hauturu.

A significant difference in the number of MCI level taxa was found in streams from the four regions ($\chi^2 = 13.25$, $P < 0.01$). Pairwise comparisons showed that streams on Hauturu and Coromandel Peninsula were significantly less species-rich than streams in Auckland and Whangarei ($P < 0.05$ for both). The two-dimensional NMS ordination based on coded abundance data (Figure 3) had a final stress value of 0.12 and was significant when tested against Monte Carlo simulations ($P < 0.01$ for both axes). The ordination clearly separated benthic macroinvertebrate communities on Hauturu from mainland communities, whose envelopes showed a degree of overlap (Figure 3). Neither axis was significantly correlated with species richness ($P > 0.05$). The multi-response permutation procedure (MRPP) also indicated significant differences between the Hauturu communities and those in each of the three mainland regions ($P < 0.01$) and significant differences between stream faunas of the Coromandel and Auckland, and Coromandel and Whangarei regions (Table 5). However, no significant difference was found between the Auckland and Whangarei stream communities. Indicator species analysis showed that five species including the caddisfly *Plectrocnemia* and the poorly known mayflies *Arachnocolus phillipsi* and *Isothraulus abditus* were largely responsible for the differences in benthic macroinvertebrate communities between Hauturu and the other three regions (Table 6). A further five species were significant indicators for Whangarei and Auckland streams but no significant indicator species were identified for Coromandel streams.

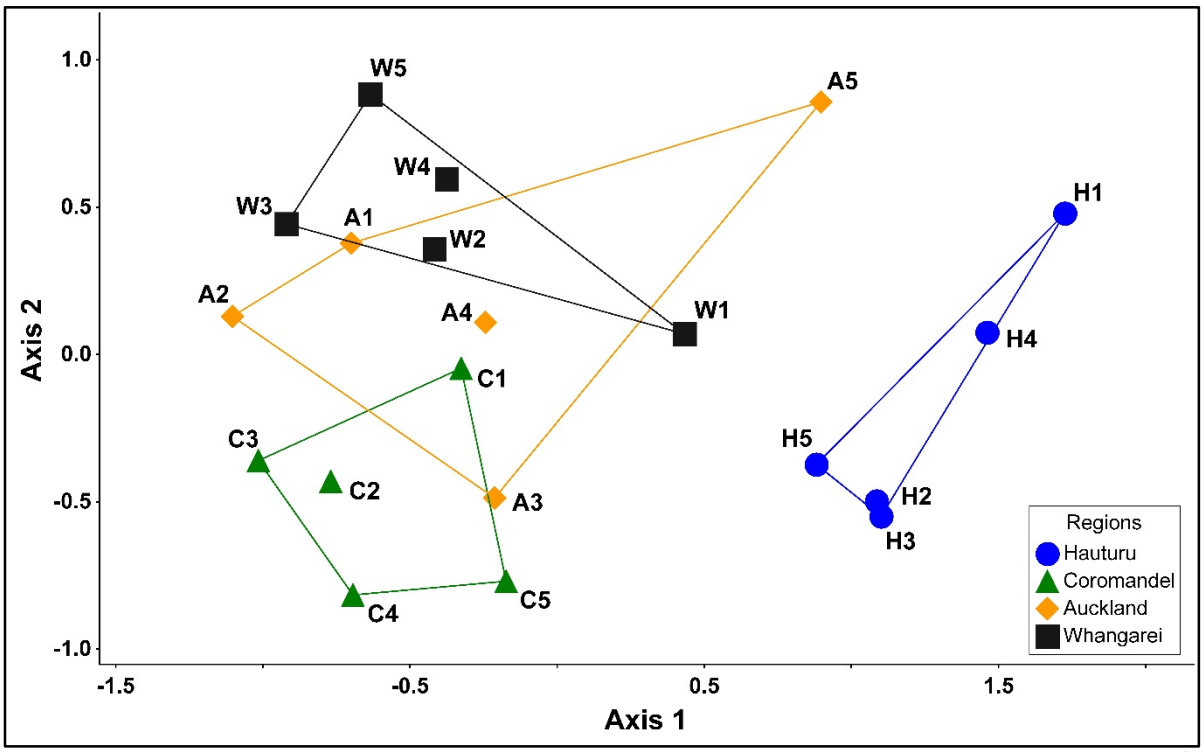

Figure 3. NMS ordination plot of benthic stream invertebrate communities based on coded abundance data (see Methods) in four regions: Hauturu, Coromandel, Auckland and Whangarei (stress = 0.12). Labels indicate individual sites that correspond to site details in Supplementary Table S1.

Table 4. Total numbers of taxa, numbers of EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa, Diptera, Mollusca, Crustacea and the top five most abundant taxa, for Hauturu and three adjacent regions of the North Island mainland. All groups are calculated at the MCI level of identification. Data derived from five streams in each region, sampled on two occasions. Top five most abundant taxa are generated from averaged coded abundances.

Taxon/Group	Hauturu	Coromandel	Auckland	Whangarei
Total taxa	46	52	81	80
EPT taxa	27	29	40	38
Diptera	8	9	17	15
Mollusca	1	2	5	6
Crustacea	1	2	7	7
Top five most abundant taxa	*Zephlebia*	*Coloburiscus*	*Hydropsyche*	*Deleatidium*
	Arachnocolus	*Deleatidium*	*Deleatidium*	*Zephlebia*
	Hydropsyche	Chironominae	*Potamopyrgus*	*Potamopyrgus*
	Deleatidium	*Potamopyrgus*	*Coloburiscus*	*Coloburiscus*
	Potamopyrgus	*Hydropsyche*	*Pycnocentrodes*	*Paracalliope*

Table 5. Pairwise comparisons of differences in benthic macroinvertebrate community composition between Hauturu, Coromandel, Auckland and Whangarei groups of streams using the multi-response permutation procedure (MRPP). Within-group homogeneity values (*A*) and their associated *P*-values are shown.

Region combination	Homogeneity (*A*)	*P*-value
Hauturu v Coromandel	0.436	0.002
Hauturu v Auckland	0.329	0.004
Hauturu v Whangarei	0.404	0.002
Coromandel v Auckland	0.155	0.01
Coromandel v Whangarei	0.245	0.005
Auckland v Whangarei	0.022	0.306

Table 6. Results of an indicator species analysis (ISA) of benthic macroinvertebrate community composition at Hauturu, Auckland and Whangarei. No indicator taxa were found for Coromandel. Only taxa with significance values < 0.05 are shown.

Taxon	*P*-value	Region
Plectrocnemia	0.003	Hauturu
Isothraulus	0.004	Hauturu
Arachnocolus	0.009	Hauturu
Polyplectropus	0.012	Hauturu
Culex	0.043	Hauturu
Limonia	0.028	Auckland
Hexatomini	0.016	Whangarei
Oligochaeta	0.021	Whangarei
Mauiulus	0.031	Whangarei
Triplectides	0.031	Whangarei

Discussion

Sixty-five aquatic invertebrate species have now been reported from Hauturu/Little Barrier Island. Thirty-nine of these were recorded by Wise (1956) and/or Winterbourn (1964), one (*Paratya curvirostris*) during fish surveys in May 2000 (NZFFD 2018) and the remaining 25 were recorded for the first time in the present study. However, three stoneflies (*Acroperla trivacuata*, *Zelandoperla* sp., and *Zelandobius* sp.), three mayflies (*Deleatidium (D.) myzobranchia*, *Zephlebia versicolor* and *Nesameletus ornatus*) and larvae belonging to the dipteran family Ceratopogonidae, all of which were recorded in 1963, were not found in 2014. The mosquito *Aedes antipodeus*, reported by Wise (1956), was not recorded in either of

the two subsequent surveys. Absence in 2014 of nymphs of at least two of the three stonefly species may be a reflection of their life cycles as Winterbourn (1966) found that adults of *A. trivacuata* and *Z. decorata* had completed emergence in January in the Auckland region. Nymphs attributed to *Z. versicolor* in 1963 may have been those of *Z. spectabilis*, a species with a broadly similar appearance described by Towns (1983) and found on Hauturu in the present study. Nymphs of *D. (D.) myzobranchia* are known from fast-flowing water (Towns & Peters 1996), conditions not present during the 2014 study; thus they may only be found further upstream during times of low flow. Nymphs of *Nesameletus ornatus* and *Zephlebia versicolor*, which have been recorded on Hauturu, as well as others such as *Z. nebulosa* and *Deleatidium (D.) angustum*, which have not, are all known from small streams and streamlets amongst organic detritus and trailing vegetation (Towns & Peters 1996; Hitchings & Staniczek 2003) and therefore could potentially occur higher in the catchments of Hauturu streams. Our sampling was confined to the lower reaches of each stream for logistical reasons. To refine the Hauturu faunal list further, small streams in the upper reaches would be valuable habitats to sample, and seepages and springs would be worth including as they have been known to contribute considerably to stream faunal lists (Collier & Smith 2006). Additionally, the inclusion of passive intercept traps (e.g. Malaise traps) could help sample species occupying habitats that are difficult to sample like seeps, and are also not attracted to lights in light traps.

No species unique to Hauturu were identified in the 2014 survey. Rather the stream fauna consisted largely of species that are typically found in the northern half of the North Island, or are common species with widespread distributions within New Zealand. Examples of the former are the mayflies *Acanthophlebia cruentata, Arachnocolus phillipsi, Isothraulus abditus, Mauiulus aquilus, Zephlebia borealis* and *Z. inconspicua*, whereas widespread species found included the stoneflies *Stenoperla prasina* and *Austroperla cyrene*, the dobsonfly *Archichauliodes diversus*, two mayflies *Deleatidium (D.) fumosum* and *D. (D.)* cf. *lillii* and the snail *Potamopyrgus antipodarum*. Our record of the nematomorph *Gordionus maori* is interesting as it is a recently described species known previously only from the South Island (Yadav et al. 2018). Sixteen of the 24 mayflies recorded by Towns (1987) from Great Barrier Island, which lies about 20 km east of Hauturu, were found on Hauturu. However, *Atalophlebioides cromwelli* and two *Austroclima* species that have been found on Great Barrier and elsewhere in the northern North Island (Towns 1987; Towns & Peters 1996) were not recorded on Hauturu.

Although streams on Hauturu are ephemeral, or intermittent, in the sense that flow is periodically disrupted so that surface water is absent or confined to pools, it was apparent that the invertebrate fauna is not characterised by ephemeral stream specialists *per se*, but rather a subset of species found in perennial and intermittent streams on the adjacent mainland. This finding is consistent with that of Storey et al. (2011) who found that the invertebrate fauna in both pools and flowing waters of ephemeral headwater streams in the Auckland region were similar to those in adjacent perennial streams. As many of the species found in pools on Hauturu inhabit riffles elsewhere, they would be

expected to colonise them on Hauturu when flowing water is present. At the time of the 1963 stream survey on Hauturu surface flow was greater than in 2014, enabling both riffles and pools to be sampled in Awaroa and Tirikakawa streams (Winterbourn 1964). Thus, of the 16 species identified by Winterbourn (1964) from riffles in those two streams, 10 were also found in pools in 1963, and 11 of them inhabited pools in 2014. These observations provide further support for the notion that numerous members of the riffle fauna move into pools when flow declines.

Species lists and presence-absence clustering indicated that the five Hauturu streams shared a large number of invertebrate species, not surprisingly, given their close proximity within native forest and their physico-chemical similarity. Parihakoakoa, the smallest stream, had the most distinctive fauna, as in 1963 (Winterbourn 1964). In 2014 the lowest dissolved oxygen concentration and high conductivity were found in its pools, and its stream channel was the most unstable as indicated by its relatively high Pfankuch index score, together suggesting that Parihakoakoa Stream may provide the most demanding physico-chemical environment for aquatic invertebrates. In particular, the low dissolved oxygen concentrations in pools during periods of low flow may prevent populations of some species from becoming established, while its smaller, less stable substrata may preclude colonisation by some species. Thus, no stoneflies or megalopterans, and only two of the 6–9 mayfly species collected from the other four streams, were found there. Nymphs of *Isothraulus abditus*, one of the two mayflies found in Parihakoakoa Stream, inhabit pools and backwaters and were found at DO concentrations as low as 1.7 g m^{-3} in a stream near Whangarei (Pohe et al. 2019). Their presence in Parihakoakoa Stream is in line with these findings and supports the suggestion that they can tolerate conditions of low dissolved oxygen. Less is known about the habitat of *Arachnocolus phillipsi*, the other mayfly found in Parihakoakoa Stream, although Towns and Peters (1979, 1996) reported that nymphs were most abundant in slow-flowing reaches of first order streams on wood, leaves and vegetation trailing in the water. Towns (1987) also found nymphs of *A. phillipsi* with those of *I. abditus* and *Zephlebia borealis* on wood and leaves in pools of first order streams on Great Barrier Island as well as on a variety of organic and inorganic substrata in larger streams.

Our comparison of the invertebrate fauna of Hauturu streams with stream faunas in three adjacent parts of the North Island mainland showed that the Hauturu streams were grouped together in ordination space, whereas streams in the Auckland, Whangarei and Coromandel regions of the mainland formed a separate, partially overlapping, cluster. Taxonomic richness of the Hauturu fauna (all streams combined) was also substantially lower than that found in the Auckland and Whangarei groups of streams (46 taxa vs 81 and 80 taxa, respectively), although only six more species were found in the Coromandel group of streams than on Hauturu. These faunal differences may largely reflect the morphology of the streams, rather than an inability of species to colonise them. Thus, Hauturu streams have limited or no low-gradient downstream reaches, in contrast to most mainland streams. Low-gradient reaches provide habitats for a number of species that have not been found on Hauturu but have

been reported in streams in the other three regions. Among them are the caddisflies *Pycnocentrodes* and *Hudsonema*, the damselfly *Austrolestes*, molluscs other than *Potamopyrgus antipodarum*, small Crustacea (Amphipoda other than *Paracalliope fluviatilis*, Copepoda, Isopoda and Ostracoda), the crayfish *Paranephrops planifrons*, leeches (Hirudinea) and nemertean worms.

Conclusions

Our survey of five streams on Hauturu resulted in the addition of 25 new species records, bringing the total number of aquatic invertebrates known from the island to 65. Stream faunas on Hauturu are dominated by the immature stages of insects including a notably high diversity of Ephemeroptera. Despite its relatively small size and island status, its isolation does not appear to have been a significant barrier to colonisation. Thus, the stream fauna of Hauturu resembles the faunas of comparable forest streams on the nearby mainland in accordance with our primary hypothesis. However, the notable absence of some species does make the fauna different, and at least in part may be a consequence of a relative absence of low-gradient habitat in the form of downstream reaches. The ephemeral nature of Hauturu streams appears to have little effect on the species composition of the invertebrate fauna, which resembles that of many ephemeral and perennial streams in the Auckland region (Storey et al. 2011) and hence is consistent with the results of an increasing number of studies elsewhere (Datry et al. 2017).

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Supplemental material

Table S1. Metadata of sampling sites used in the study. Available for download (655 KB) at: https://www.tandfonline.com/doi/suppl/10.1080/03014223.2019.1576214/suppl_file/tnzz_a_1576214_sm9510.pdf.

Figure S2. Photos of select Hauturu streams with almost no surface flow (taken mid-January 2014). Available for download (6.2 MB) at: https://www.tandfonline.com/doi/suppl/10.1080/03014223.2019.1576214/suppl_file/tnzz_a_1576214_sm9511.pdf.